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TITLE: A Structure Based, Solid-Phase Synthesis Approach to the Development of Novel Selective Estrogen Receptor Modulatory Steroids

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**13. Abstract** *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*
The overall objective of this project was the development of new chemotherapeutic agents for the treatment of hormone-responsive breast cancer. The specific aims included: (1) the preparation of polymer-bound steroidal starting materials; (2) elaboration via acylation/amidation reactions; (3) biological evaluation of the new compounds; and (4) identification of leads for subsequent optimization. We prepared the polymer-bound estradiol derivatives and prepared several preliminary series of derivatives. We compared the solid-phase and solution-phase methods and concluded that at this time solution-phase chemistry was more reliable and subsequent chemistry used this approach. Several series of 17-α-(substituted aryl)vinyl estradiols were evaluated for ER-hormone binding domain affinity and in vivo efficacy. Most compounds retained significant ER affinity (5-160% of estradiol) and all compounds so far were agonists. Evaluation using molecular modeling and molecular dynamics indicated that the arylvinyl substituents were interacting in the key helix-12 region. In summary, synthetic approaches to potent ER-ligands have been developed and the evaluation results indicated that further derivatives may lead to desired objectives. Follow on studies are now in progress.

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## Table of Contents

INTRODUCTION:

The overall objective of this project was the development of new chemotherapeutic agents for the treatment of hormone-responsive breast cancer. The research approach envisioned the use of solid-phase organic synthesis to generate libraries of estradiol derivatives that would display estrogen-receptor modulatory properties. Evaluation by receptor binding and cell proliferation assays would identify lead compounds that would subsequently be modified to optimize potency and selectivity. During the execution of the project we developed the solid-phase synthetic methods and prepared several series of estradiol derivatives. However, we found that this approach was not superior to solution-based methods at this time and subsequent syntheses used the latter approach. We evaluated the several series of substituted arylvinyl estradiols and found that the position, as well as the properties, of the substituent played a siginificant role in the binding and potency of the compounds. Most of the products were more potent than the unsubstituted phenylvinyl estradiol but not as potent as estradiol itself. The cell proliferation assay was unreliable and replaced by the uterotrophic growth assay which indicated that all of the new compounds were full agonists. We examined the compounds using conformational analysis (NMR) and molecular modeling (docking) to determine binding effects. The results suggested that the 17$\alpha$-(substituted phenyl)vinyl group was accommodated by the estrogen receptor such that the helix-12 would still achieve an agonist conformation. However, further extensions at that position may still lead to the desired modulatory effect and syntheses of aminomethyl- and carboxy-phenyl derivatives were undertaken. The results of these studies are not yet available.

This project has demonstrated the feasibility of this approach in generating novel and potent estrogen receptor ligands. The compounds demonstrate high affinity, and with further structural modification may lead to the desired modulatory effects. The results have generated several publications and presentations, and have produced new projects in related areas that have received extramural support.

BODY:

The proposal identified five (5) tasks to be completed. They were:
1. Synthesis of the polymer-bound estradiols
2. Synthesis of structure-based libraries
3. Determination of biological properties
4. Assessment of structure-activity relationships
5. Synthesis of second library

Task 1. Synthesis of polymer-bound estradiols.

The synthetic approach to the polymer-bound estradiols was achieved by coupling the requisite stannylviny estradiol to the carboxylated resin. These results were described in the publication by Lee, et al.(1), and in her doctoral thesis (2). To prepare the subsequent functionalized aminomethyl-phenylvinyl and carboxyphenylvinyl estradiols on the carboxylated resin required additional steps. The protected or unprotected 3-aminomethylphenyl iodide was coupled to the resin bound stannylvinyl estradiol using Stille coupling procedures. Careful deprotection of the aminomethyl group gave the desired polymer-bound aminomethylphenyl vinyl estradiol, albeit in modest yields. Trimethylsilylethyl 4-iodobenzoate was coupled to the stannylvinyl estradiol, also using the Stille reaction method. Deprotection with tetrabutylammonium fluoride gave the carboxyphenyl vinyl estradiol, also in modest yields. The compounds were characterized by cleavage from the resin and analyzed using NMR and elemental analysis. Therefore, the first task was achieved.

Task 2. Synthesis of the first libraries.

This task was conducted in parallel with other ongoing projects directed toward estrogenic ligands. The initial work involved comparing the solid-phase and solution-based methods for preparing mono-substituted phenylvinyl estradiols. We prepared a variety of 2-,3-,4-substituted phenyl vinyl estradiols with both cis (Z) and trans (E) stereochemistry. In general, yields for the two methods were comparable for the simple mono-substituted compounds. For the other series- aminoacylated and carboxamido phenylvinyl estradiols- solution based methods were preferable. Although solid-phase chemistry gave the target compounds, as described in Hanson, et al., (3.4), the process was less efficient than solution-based methods. We are currently developing the methods to employ parallel synthesis for the combinatorial chemistry. The products generated in this task were characterized by NMR and elemental analysis. Therefore, we have successfully completed the aims of the second task.

Task 3. Biological evaluation of the new compounds.

This task involved the evaluation of the new compounds as ligands for the estrogen receptor hormone binding domain (ER-HBD) and determination of their properties as agonists/antagonists. Our initial assay system utilized a competitive binding

assay with the ER-HBD overexpressed by BL21 cells, a standard method for determining relative binding affinities(RBA) of estrogenic ligands. Using this procedure, we have

| R= | 2- | 3- | 4- |
|---|---|---|---|
| $CH_3$ | 57/60 | 12/12 | 10/18 |
| $CF_3$ | 71/190 | 20/22 | 7/6 |
| $CO_2CH_3$ | 30/23 | 17/26 | 16/17 |
| F | 16/15 | 16/20 | 24/37 |
| OH | 24/46 | 138/91 | 21/25 |
| $OCH_3$ | -/- | -/- | 36/32 |
| CN | -/- | -/- | 9/27 |
| $CH_2OH$ | 7/11 | -/- | 2/2 |
| $COCH_3$ | -/- | -/- | 53/60 |
| $CO_2H$ | 0.5/0.7 | 1.3/1.6 | 0.9/1.3 |
| $NO_2$ | -/- | 25/42 | -/- |
| $CH_2NH_2$ | -/- | 19/18 | -/- |

H = 16/9
Estradiol = 100/100

Table 1A. Relative Binding Affinities (RBA) at 2°C/25°C for E-(trans)-Substituted Phenyl Vinyl Estradiols

| R= | 2- | 3- | 4- |
|---|---|---|---|
| $CH_3$ | 30/27 | 40/45 | 10/9 |
| $CF_3$ | 6/26 | 30/60 | 5/9 |
| $CO_2CH_3$ | 26/49 | 6/12 | 72/57 |
| F | 30/73 | 14/16 | 21/33 |
| OH | 48/57 | -/- | 12/25 |
| $OCH_3$ | -/- | -/- | 20/39 |
| CN | -/- | -/- | 20/12 |
| $CH_2OH$ | 123/114 | -/- | 2/2 |
| $CO_2H$ | 2/3 | -/- | -/- |
| $NO_2$ | -/- | -/- | -/- |
| $CH_2NH_2$ | -/- | 88/82 | -/- |

H = 57/66
Estradiol = 100/100

Table 1B. Relative Binding Affinities (RBA) at 2°C/25°C for Z-(cis)-Substituted Phenyl Vinyl Estradiols

analyzed over 75 compounds, including those prepared in this project. From the series of compounds generated in Task 2, most of the compounds had higher RBA values than the parent unsubstituted phenyl vinyl estradiol (RBA= 9-16), but less than estradiol (RBA= 100) (Table 1A,B). Because most of the compounds demonstrated significant affinity for the ER-HBD, all of the compounds were considered for evaluation in the efficacy assays.

Initially we considered the MCF-7 cell proliferation assay to determine the ability of the compounds to stimulate or inhibit cell growth. (We would subsequently do [H-3]-thymidine incorporation as a further measure of cell inhibition.) However, the assay methods did not provide sufficient reproducibility and we eventually abandoned the MCF-7 proliferation test. Interestingly, some compounds that at low doses stimulated cell proliferation, at higher doses were cytotoxic to the MCF-7 cell lines. Whether this was ER-mediated was not determined, but it will be evaluated in the future in other projects. We subsequently used the immature rat uterotrophic growth assay to evaluate efficacy.

| **Substituent** | **Isomer** | **$ED_{50max}$ (nmole)** | **RBA 2°C/25°C** |
|---|---|---|---|
| Methyl | Z-ortho | 0.16 | 30/27 |
| | E-ortho | 3.0 | 25/27 |
| | Z-para | 15.4 | 10/9 |
| | Z-meta | 11.7 | 40/45 |
| | E-para | 5.4 | 10/18 |
| | E-meta | 8.8 | 12/12 |
| Methyl ester | Z-para | 2.5 | 72/57 |
| | Z-ortho | 24 | 26/49 |
| | E-ortho | 35 | 30/23 |
| | Z-meta | 76 | 6/12 |
| | E-meta | 200 | 17/26 |
| | E-para | 240 | 16/17 |
| Trifluoromethyl | E-ortho | 0.42 | 48/223 |
| | Z-para | 1.2 | 5/9 |
| | Z-meta | 1.5 | 30/60 |
| | Z-ortho | 12 | 6/26 |
| | E-para | 13 | 6/8 |
| | E-meta | 25 | 38/75 |
| Fluoro | Z-ortho | 9.7 | 30/73 |
| | Z-meta | 2.5 | 14/16 |
| | Z-para | 3.2 | 21/33 |
| | E-ortho | 8.3 | 16/15 |
| | E-meta | 10 | 16/20 |
| | E-para | 70 | 24/37 |
| Estradiol | | 0.08 | 100/100 |

Table 2. Comparison of uterotrophic activities and RBAs of Substituted Phenyl Vinyl Estradiols

This was a time-consuming and expensive assay that was run in parallel with other ongoing projects. Essentially, we would run an entire series of six isomers and standards simultaneously (280-300 rats) to have internally consistent results. Eventually we were able to obtain results for six series of compounds (Table 2). Examples of the assay curves are provided in the Appendix. Uterotrophic growth assays of test compounds versus estradiol were run to identify if any compounds were antagonists, however, all the results were negative for antiestrogenicity.

Given that the objectives for this task were to develop the assay systems and evaluate the compounds prepared in Task 2, we have fulfilled those objectives.

TASK 4: Structure-Activity Relationships

In this task we attempted to find relationships between the structural features and the biological properties using conformational analysis and molecular modeling. An initial analysis of the structures and the RBA values provided several findings. In general, the cis (Z) isomers were more potent binding agents than the corresponding trans (E) isomers. Within each series, there were different effects for each substituent depending upon whether it was in the 2-,3-, or 4-position of the phenyl ring, i.e., each position had its own SAR. In general, the 2-isomers were more potent than the 3- or 4- isomers, but there were exceptions. In some cases, the 2-E-isomers were more potent than the 2-Z-isomers. We consequently analyzed this effect using high field NMR conformational analysis. These studies were described in two papers by Sebag, et al., (5,6). The indicated that the Z-isomers exhibit torsional rotation around the vinyl-phenyl junction for all substitution patterns whereas this effect is only present in the 2-E-isomers. We used these findings in our subsequent molecular modeling studies in which we docked the 4-substituted phenyl vinyl estradiols with the ERα-HBD. In this study, we calculated the binding energy of each complex and compared it to the observed RBA value. For a series of 12 compounds, 10 of 12 complexes gave a linear correlation with an $R^2$= 0.945. The graph of these data are provided in the Appendix.The formulation of the model that gave that correlation required the development of parameters not available in the software package and took significant time and effort. Nevertheless, it is now being applied to the 3- and 4-E-phenyl vinyl estradiols.

We also evaluated the compounds prepared from the aminomethyl and carboxy phenyl vinyl series. In general the results were disappointing. The 3-aminomethyl phenyl vinyl estradiol displayed high affinity (RBA= 18-19), but the acylated derivatives were almost an order of magnitude lower (RBA= 3-5) except for the bromoacetyl derivative which had an RBA = 35. Similar results were obtained for the 4-carboxamido phenyl vinyl estradiols. While the 4-methoxycarbonyl compound was a reasonable ligand (RBA = 26), the N-methyl, N-benzyl and N-methoxycarbonylbenzyl amides were an order of magnitude lower (RBA = 3-4). These results are described in the manuscript by Hanson, et al. (3). This is still significant affinity compared to many estrogenic ligands, but it is still low compared to our lead compounds. We have reported the preliminary results of the in vitro binding and in vivo activity in two manuscripts (7,8). Therefore the aims of this task have been largely completed.

TASK 5: Synthesis and evaluation of second library.

Based upon the results of the binding studies and the synthetic methods development, we have undertaken the preparation of the second library of ligands. We have elected to use solution-based chemistry to prepare the target compounds. Our target set includes the 4-substituted benzoylaminomethyl phenyl vinyl estradiols and the 4-substituted benzylaminocarbonyl phenyl vinyl estradiols. We prepared the requisite intermediates by simple acylation chemistry with the 4-iodobenzylamine or 4-iodobenzoyl chloride. The products were purified by recrystallization and characterized. Stille coupling with either stannylvinyl estradiol or its 3-acetyl derivative gave the target compounds in good (>80%) yields. This was an improvement over the solid-phase method, although it did require one-by-one synthesis. The compounds were characterized by NMR and elemental analysis. The purified compounds have been submitted for biological evaluation. Therefore, the aims of this task have not yet been completed.

KEY RESEARCH ACCOMPLISHMENTS

- Developed and applied solid-phase synthesis methods for 17α-Substituted Phenyl Vinyl Estradiols
- Evaluated several series of substituted phenyl vinyl estradiols as ER-ligands and agents
- Identified members of the series as leads for ER-ligand / agent development
- Correlated affinity of 4-substituted phenyl vinyl estradiols with binding energy via molecular modeling
- Applied results to development of related approaches for breast cancer hormone therapy

REPORTABLE OUTCOMES;

Manuscripts-published/accepted/submitted


1. Lee, C.Y. and Hanson, R.N. Solid phase synthesis of 17α-E/Z-(X-phenyl)-vinyl estradiols using the Stille coupling reaction. Tetrahedron 2000; 56: 1623-1629.
2. Hanson, R.N. Synthesis of Auger electron-emitting radiopharmaceuticals. Curr. Pharm. Design, 2000; 6: 1457-1468.
3. Sebag, A.B., Friel, C.J., Hanson, R.N., and Forsyth, D.A. Conformational studies on (17α,20Z)-21-(X-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols using 1D and 2D NMR spectroscopy and GIAO calculations from C-13 shieldings. J.Org. Chem., 2000; 65: 7902-7912.
4. Sebag, A.B., Lee, C.Y., Hanson, R.N., and Forsyth, D.A. Conformational studies on (17α,20E)-21-(X-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols using 1D and 2D NMR spectroscopy and GIAO calculations from C-13 shieldings. Mag. Res.Chem. 2002 (Accepted)
5. Hanson, R.N., Lee, C.Y., Friel, C., Hughes, A. DeSombre, E.R. Evaluation of 17α-E-(Tifluoromethylphenyl)vinyl estradiols as novel estrogen receptor ligands. Steroids 2002 (Accepted)

6. Hanson, R.N., Lee, C.Y., DeSombre, E.R., Hughes, A. Solid-phase synthesis of a series of 17α-(4-carboxamidophenyl)vinyl estradiols and their evaluation as estrogen-receptor ligands. Bio-org. Med. Chem. Letters 2002 (Accepted)
7. Hanson, R.N., Lee, C.Y., Friel, C.J., Dilis, R., DeSombre, E.R., Hughes, A. Synthesis and evaluation of (17α,20E)-21-(4-substituted-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols as probes for the estrogen receptor-alpha (ERα) hormone binding domain. J. Med. Chem. 2002 (Submitted)

Abstracts/presentations

1. Hanson, R.N., Friel, C.J., Lee, C.Y., DeSombre, E.R., Hughes, A. Design, synthesis and evaluation of 17α-(E/Z)-arylvinyl estradiols as (anti)estrogens. Gordon Research Conference-poster. Medicinal Chemistry. August 2001

Patents - Awarded/Pending

1. Hanson, R.N., Friel, C., Lee, C.Y. Preparation of novel steroidal antiestrogens and antiandrogens for the treatment of hormone-responsive disorders. WO 2001098322.

Training- Theses: Ph.D./M.S.

1. Choon Young Lee. Ph.D. Medicinal Chemistry (2000)
2. Rachel Gershman, M.S. Chemistry (2002)

Funding-Awarded/Pending

1. **USAMRMC-DAMD17-00-100384** 7/1/00-6/60/03 $309,874 5% Effort
   Role: Principal Investigator
   "Solid-Phase Combinatorial Approach to Estradiol-Tamoxifen/Raloxifene Hybrids: Novel Chemotherapeutic/Prophylactic Selective Estrogen Receptor Modulators (SERMs)
2. **Massachusetts Department of Public Health** 1/1/01-12/31/03
   $161,334 0% Effort (Suspended pending restoration of funding)
   Role: Principal Investigator
   "A Solid Phase Synthesis Approach to the Development of Chemotherapeutic Agents for the Treatment of Hormone Responsive Prostate Cancer"
3. **National Institutes of Health 1 RO1 CA89488-01** 7/01/01-6/30/06
   $190,714 5%Effort
   Role: Co-investigator (Geoffrey Greene, P.I.)
   "Development and Characterization of Novel SERMs"
4. **National Institutes of Health 1 RO1 DK-61084-01** 9/30/02-9/29/05
   $200,000 5% Effort
   Role: Co-investigator (Shuk-Mei Ho, P.I.)
   "Estrogen Receptor-beta and Prostate Function"

CONCLUSIONS:

The project has resulted in the successful completion of most of the specific aims listed in the original proposal. The investigators developed a solid-phase synthetic approach to the preparation of libraries of novel estrogenic ligands. Ultimately, this approach may be the method of choice for the synthesis of the targeted amino acid derivatives of the 17α-(aminomethyl/carboxy-phenyl)vinyl estradiols. However, for the simpler derivatives prepared in this project, solution-based methods were simpler and more reliable. Because identification of potential therapeutic agents was the objective of the project, and not method development, we used the solution-based approach for the preparation of the second library currently undergoing biological evaluation. Similarly, we modified our bioanalytical methods in order to achieve consistency in the data. MCF-7 cell proliferation and thymidine incorporation assays were replaced by a uterotrophic growth assay. We developed sophisticated conformational analysis and molecular dynamics methods to interpret the interactions between our compounds and the estrogen receptor. The studies indicated that the initial series of compounds were accommodated by the ligand binding site in an agonist rather than an antagonist mode. We are performing further modeling studies to identify substituents that may convert agonists to antagonists within this family of compounds.

Future activities related to this project should include the following aims: 1. Improved preparation of the aminomethyl- and carboxy-phenyl vinyl estradiols bound to the resin and reactions thereon; 2. More extensive molecular modeling to identify groups that would generate antagonist binding modes in order to reduce the number of compounds to be synthesized; and 3. Improved bioanalytical methods to expedite receptor binding and efficacy assays.

This research program has many possibilities for success, but it requires funding beyond the scope of the BCRP. As an applied research project it falls neither into NIH nor NSF funding patterns, and it is longer term than most private foundations are willing to support. I will continue to pursue federal support for this project but the aims will be necessarily more limited than I would prefer.

REFERENCES:


1. Lee, C.Y. and Hanson, R.N. Solid phase synthesis of 17α-E/Z-(X-phenyl)-vinyl estradiols using the Stille coupling reaction. Tetrahedron 2000; 56: 1623-1629.
2. Choon Young Lee. Ph.D. Medicinal Chemistry (2000)
3. Hanson, R.N., Lee, C.Y., DeSombre, E.R., Hughes, A. Solid-phase synthesis of a series of 17α-(4-carboxamidophenyl)vinyl estradiols and their evaluation as estrogen-receptor ligands. Bio-org. Med. Chem. Letters 2002 (Accepted)
4. Hanson, R.N. Unpublished data. Manuscript in preparation for Bio-org. Med. Chem. Letters
5. Sebag, A.B., Friel, C.J., Hanson, R.N., and Forsyth, D.A. Conformational studies on (17α,20Z)-21-(X-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols using 1D and 2D NMR spectroscopy and GIAO calculations from C-13 shieldings. J.Org. Chem., 2000; 65: 7902-7912.
6. Sebag, A.B., Lee, C.Y., Hanson, R.N., and Forsyth, D.A. Conformational studies on (17α,20E)-21-(X-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols using 1D and 2D

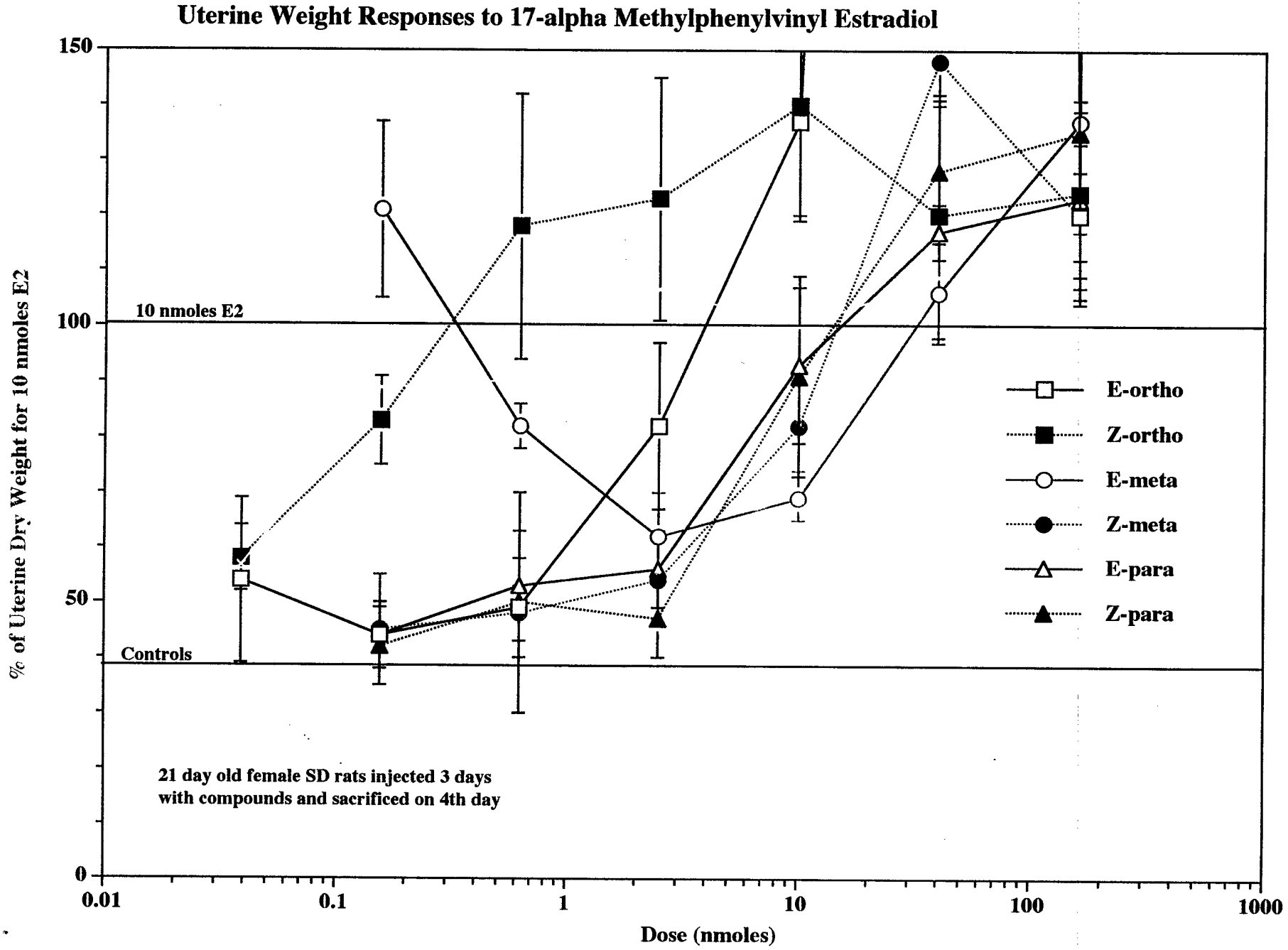
NMR spectroscopy and GIAO calculations from C-13 shieldings. Mag. Res.Chem. 2002 (Accepted)
7. Hanson, R.N., Lee, C.Y., Friel, C., Hughes, A. DeSombre, E.R. Evaluation of 17α-E-(Tifluoromethylphenyl)vinyl estradiols as novel estrogen receptor ligands. Steroids 2002 (Accepted)
8. Hanson, R.N., Lee, C.Y., Friel, C.J., Dilis, R., DeSombre, E.R., Hughes, A. Synthesis and evaluation of (17α,20E)-21-(4-substituted-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols as probes for the estrogen receptor-alpha (ERα) hormone binding domain. J. Med. Chem. 2002 (Submitted)

APPENDICES:

Assays curves (2) for uterotrophic growth assay. These illustrate the effect of dose and compound structure on biological response.
Figure of Anti-estrogenicity assy. This illustrated that none of the compounds were antagonists in this assay.
Graph of calculated binding energy versus observed RBA values (1). This illustrates the linear relationship between the two parameters using the molecular model developed in our laboratory.

Copies of manuscripts cited in Reportable Outcomes (7).

Copies of abstracts (4).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Dose (nmoles)
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Z-ortho
E-meta
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Z-para
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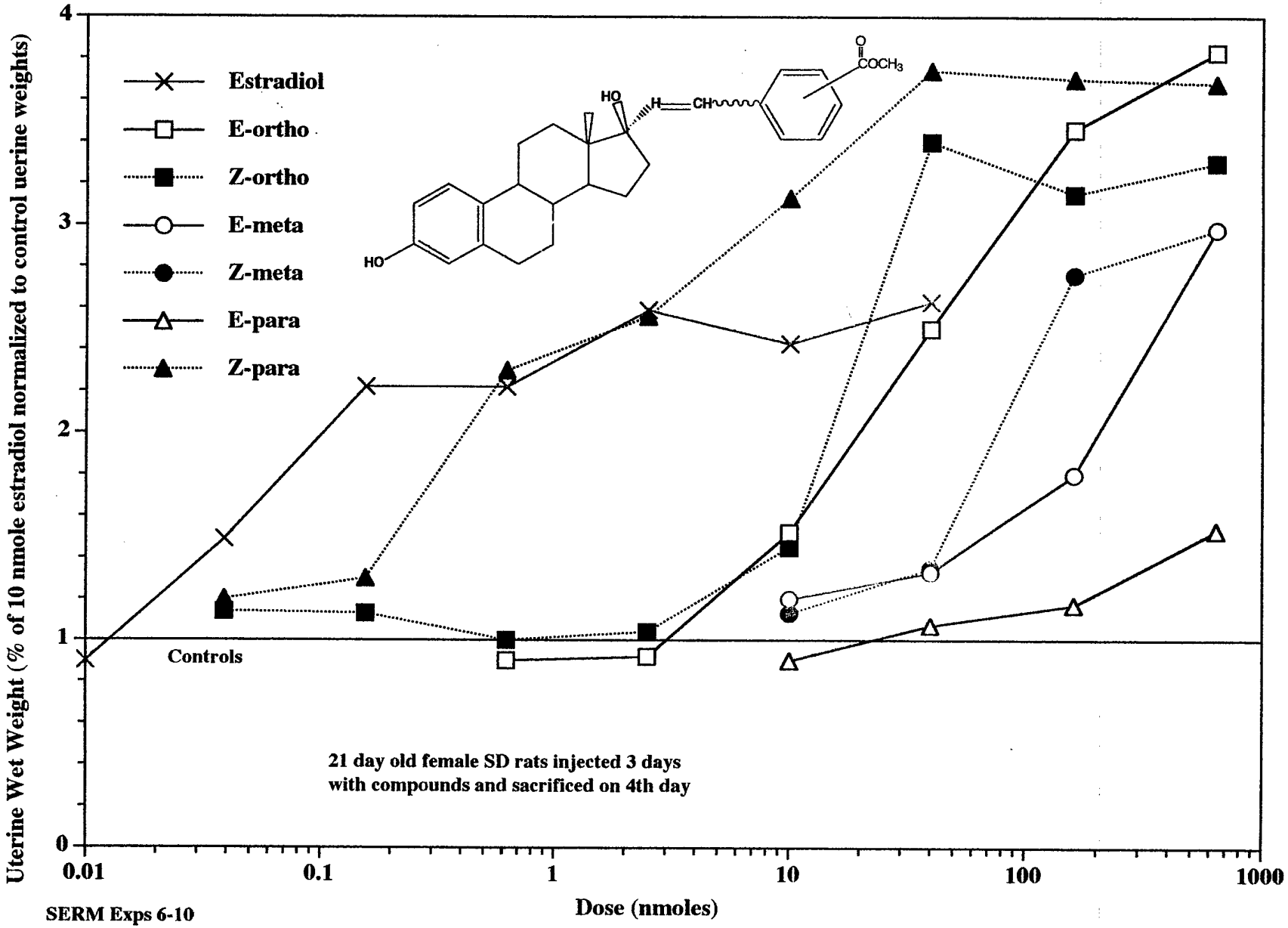
with compounds and sacrificed on 4th day

# Uterine Weight Responses to Methyl Esters of 17-alpha Phenylvinyl Estradiol

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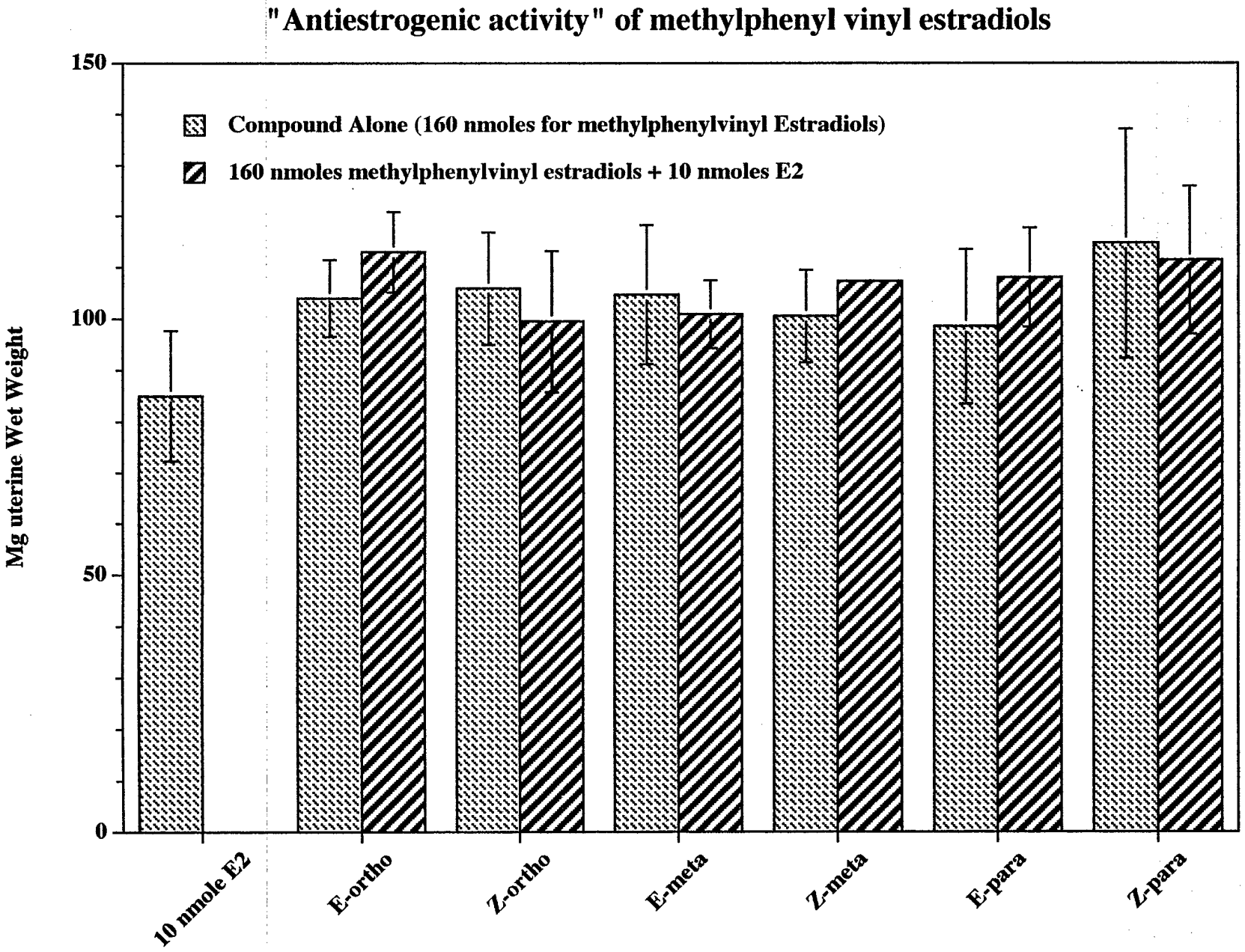
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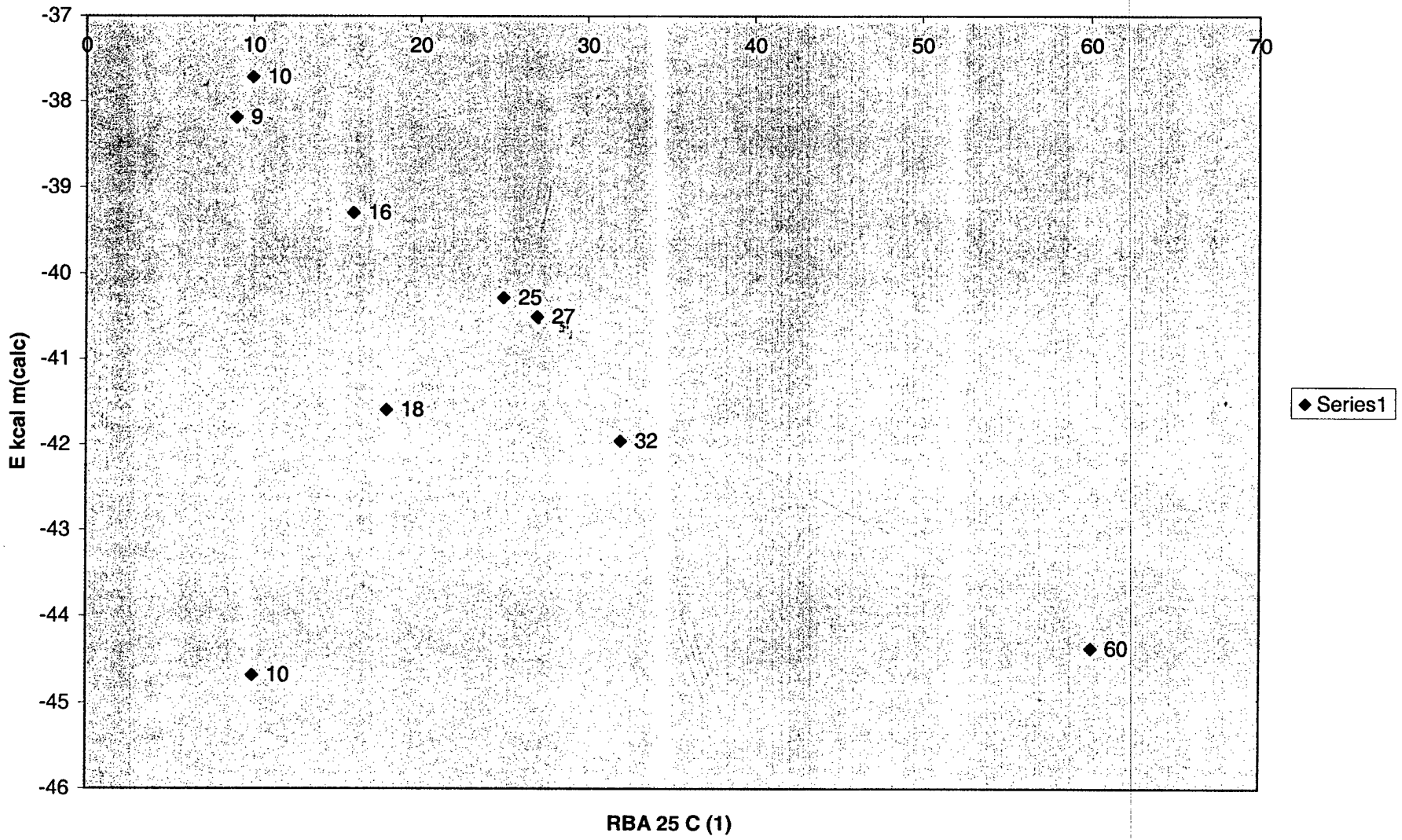
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9
16
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Series1

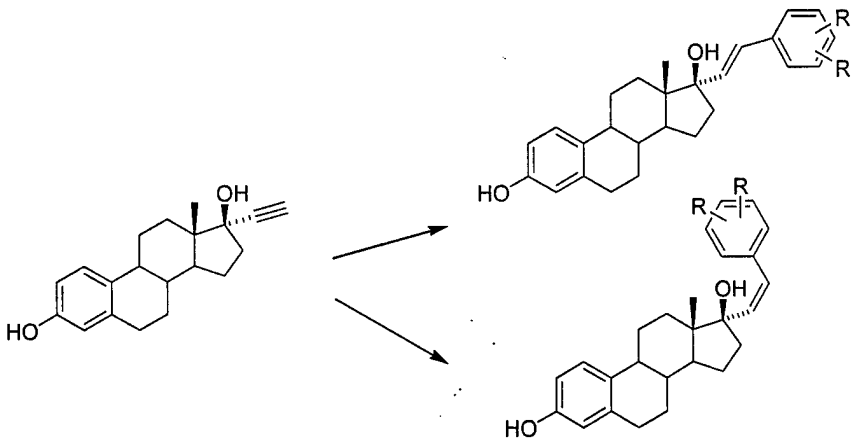
Design, Synthesis and Evaluation of 17α-(E/Z)-Arylvinyl Estradiols as (Anti)Estrogens

Robert N. Hanson, Carolyn J. Friel, Choon Young Lee, Eugene DeSombre, and Alun Hughes

Department of Chemistry, Northeastern University, Boston, MA and

The Ben May Institute for Cancer Research, The University of Chicago Medical Center, Chicago, IL

Breast cancer is the most common cancer diagnosis in the female population with an estimated 181,000 new cases per year in the United States. Approximately 60% of these cases have hormone-dependent (responsive) breast cancer, defined as containing estrogen receptors and requiring the presence of circulating estrogens for the maintenance of tumor growth. Our research has focused on developing agents that can selectively block the stimulation of tumor growth while maintaining collateral estrogen effects, i.e., to prepare a selective estrogen receptor modulator (SERM). While most efforts to date have concentrated on nonsteroidal agents, our strategy is based on structural modifications of potent steroidal compounds originally synthesized as imaging agents. The project has examined synthetic methods, extension to solution and solid phase combinatorial chemistry, conformational analysis of ligands, and molecular modeling. Preliminary results have yielded promising leads for agonists, antagonists and mixed agonist-antagonists.




This work has been supported by awards from the Public Health Service ( 1RO1 CA 81049) and the Department of Defense (DAMD17-99-9333 and DAMD-17-00-1-0384).

Preparation and Evaluation of Isomeric Series of Substituted Phenylvinyl Estradiols

Robert N. Hanson[1], Choon Young Lee[1], Carolyn Friel[1], Robert Dilis[1], Eugene R. DeSombre[2], Alun Hughes[2]

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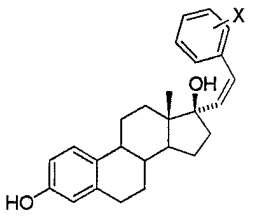
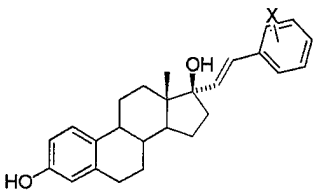
2. The Ben May Institute for Cancer Research, The University of Chicago, 5841 S. Maryland Avenue, Chicago, IL 60637

As part of our program to develop novel probes for the estrogen receptor (ER), we synthesized a series of 17α-(E/Z)-(o-,m-,p-trifluoromethylphenyl)vinyl estradiols. Preliminary binding assays indicated that stereochemistry around the double bond and position of substitution influenced the relative binding affinity (RBA). We subsequently prepared additional series having different substituents on the phenyl ring and determined the RBA values. Six complete series have now also been evaluated for their in vivo uterotrophic activity. The influence of the substituent on affinity and efficacy was examined by conformational analysis using NMR and molecular modeling.




Z-17α-(X-phenyl)vinyl estradiols




E-17α-(X-phenyl)vinyl estradiols

This research was supported in part by RO1-CA-81049, DAMD-17-99-9333, and DAMD-17-00-100384.

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# Evaluation of 17-alpha-(X-phenyl)vinyl estradiols as estrogen receptor agonists

**Robert N. Hanson**[1], Carolyn Friel[1], Choon Young Lee[1], Robert Dilis[1], Eugene R. DeSombre[2], and Alun Hughes[2]. (1) Department of Chemistry, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, Fax: 617-373-8795, r.hanson@neu.edu, (2) Ben May Institute for Cancer Research, University of Chicago

As part of our program to develop novel probes for the estrogen receptor (ER), we synthesized a series of 17 -(E/Z)-(o-,m-,p-trifluoromethylphenyl)vinyl estradiols. Preliminary binding assays indicated that stereochemistry around the double bond and position of substitution influenced the relative binding affinity (RBA). We subsequently prepared additional series having different substituents on the phenyl ring and determined the RBA values. Six complete series have now also been evaluated for their in vivo uterotrophic activity. The influence of the substituent on affinity and efficacy was examined by conformational analysis using NMR and molecular modeling.

This research was supported in part by RO1-CA-81049, DAMD-17-99-9333, and DAMD-17-00-100384.

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**Abstract DAMD17-99-1-9333:**

A Structure-Based Solid-Phase Synthesis Approach to the Development of Novel Selective Estrogen Receptor Modulating Steroids

**Principal Investigator:** Robert N. Hanson, Ph.D.

**Objective:** The project undertook the development of new steroidal chemotherapeutic agents utilizing a solid-phase synthesis approach.

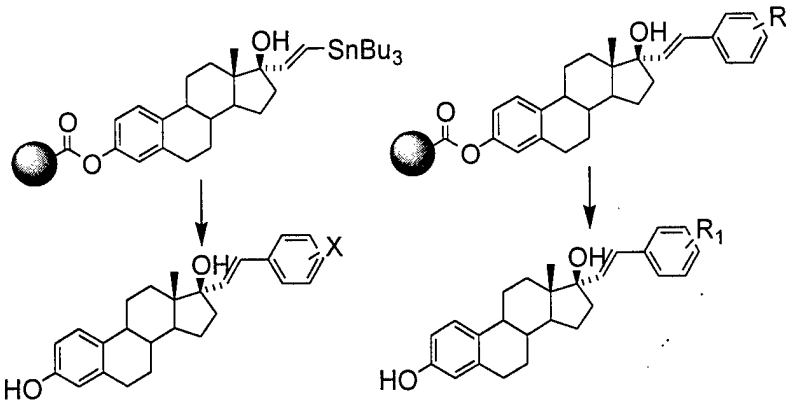
**Specific Aims:**

1. Preparation of resin bound stannylated steroid intermediate.
2. Synthesis and characterization of mono-substituted estrogens, derivatives of aminomethylphenyl and carboxamidophenyl estrogens.
3. Evaluate new derivatives as estrogen receptor binding agents.
4. Prepare second generation estradiol derivatives based on biological results.

Results:

The results of this project are summarized in the following figures. We were able to prepare the stannylated estradiol and link it to a carboxy resin via the 3-hydroxyl group. Initially we demonstrated the feasibility of the Stille coupling approach using mono-substituted aryl iodides. Subsequently we prepared the resin bound 3-aminomethyl phenylvinyl estradiol and converted it to a series of 3-acylaminomethyl phenylvinyl estradiols. Similarly we prepared the 4-carboxy phenylvinyl estradiol and converted it to a series of 4-carboxamido phenylvinyl esradiols. These series were evaluated for their affinity for the estrogen receptor (alpha)-ligand binding domain (ERα-LBD). Relative binding affinities (RBA) were generally significantly lower than estradiol and the underivatized compounds but still demonstrated estrogenic effects. Based upon these results we have undertaken the synthesis of analogs utilizing a modification (convergent synthesis) of the initial approach.

**Work During Years 1and 2**



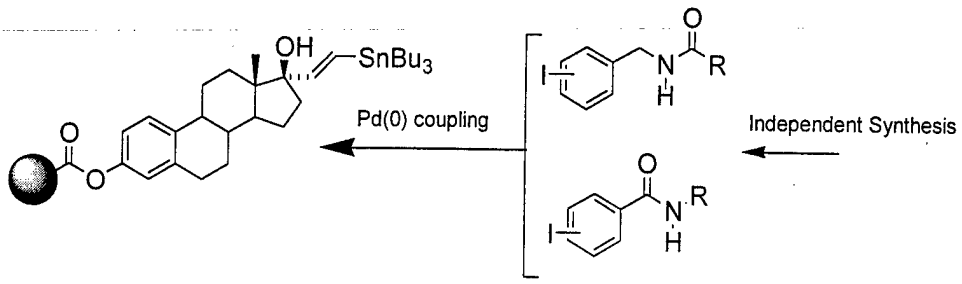

Series 1, X= mono functional group

Series 2n R1=3-Acylaminomethyl-
Series 3, R1=4-Carboxamido-

The biological data were correlated with the structures using NMR conformational analysis and by molecular modeling. The NMR studies indicated that the derivatives existed in solution in an equilibrium between two low energy conformers. These conformers were among those identified by molecular modeling and were docked

with the crystal structure for the ER-LBD. The model suggested that the 17α-substituents were tolerated by the receptor and were in a position to affect the orientation of the key helix-12 of the protein. These finding supported our further synthetic efforts toward preparing estrogen receptor antagonists and modulators.

**Future series**




Publications:


110. Lee, C.Y. and Hanson, R.N. Solid phase synthesis of 17α-E/Z-(X-phenyl)-vinyl estradiols using the Stille coupling reaction. Tetrahedron 2000; 56: 1623-1629.

111. Sebag, A.B., Friel, C.J., Hanson, R.N., and Forsyth, D.A. Conformational studies on (17α,20Z)-21-(X-phenyl)-19-norpregna-1,3,5(10),20-tetraenbe-3,17β-diols using 1D and 2D NMR spectroscopy and GIAO calculations from C-13 shieldings. J.Org. Chem., 2000; 65: 7902-7912.

112. Sebag, A.B., Lee, C.Y., Hanson, R.N., and Forsyth, D.A. Conformational studies on (17α,20E)-21-(X-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols using 1D and 2D NMR spectroscopy and GIAO calculations from C-13 shieldings. Mag. Res.Chem. 2002 (Accepted)

113. Hanson, R.N., Lee, C.Y., DeSombre, E.R., Hughes, A. Solid-phase synthesis of a series of novel 17α-(3-acylaminomethylphenyl)vinyl estradiols. Bio-org. Med. Chem. Letters (Submitted)

114. Hanson, R.N., Lee, C.Y., DeSombre, E.R., Hughes, A. Solid-hase synthesis of a series of 17α-(4-carboxamidophenyl)vinyl estradiols and their evaluation as estrogen receptor ligands. Bio-org. Med. Chem. Letters (Submitted)

115. Hanson, R.N., Lee,C.Y., Friel, C., Hughes, A., DeSombre, E.R. Evaluation of 17α-E-trifluoromethylphenylvinyl estradiols as novel estrogen receptor ligands. Steroids (Submitted)

Pergamon

Tetrahedron 56 (2000) 1623–1629

TETRAHEDRON

# Solid Phase Synthesis of 17α-*E*/*Z*-(X-Phenyl)-Vinyl Estradiols Using the Stille Coupling Reaction

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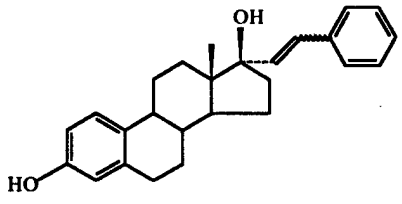
Received 23 July 1999; accepted 22 December 1999

**Abstract**—As a continuation of our program to develop probes for the hormone binding domain (HBD) of the estrogen receptor (ER), we designed a series of novel 17α-*E*/*Z*-(X-phenyl)-vinyl estradiols. Based upon our experience with solution chemistry we applied solid phase synthesis using carboxylated resins to synthesize the new compounds. The Stille coupling reaction permitted the introduction of a variety of functional groups and positional isomers on the terminal phenyl group. Subsequent cleavage from the resin generated a series of novel estradiol derivatives. 

## Introduction

As a part of our ongoing program to design and develop new therapeutic agents for the treatment of breast cancer, we have focused on new steroidal derivatives that interact at the hormone binding domain (HBD) of the estrogen receptor (ER). While many of our initial studies confirmed the established estrogen receptor structure activity relationships, derivatives with the *E*- and *Z*-X-vinyl group at the 17α-position particularly demonstrated unusual properties.[1] Further explorations with phenylvinyl (I) and phenylselenovinyl (II) estradiol suggested that receptor affinities comparable to estradiol itself could be maintained in spite of the apparent steric bulk of the 17α substituent.[2] Recent publications of the crystal structure of the liganded HBD of the ER[3] suggested that the 17α groups project into a region that may accommodate significant steric tolerance. We have elected to develop new estradiol derivatives that could exploit that tolerance.

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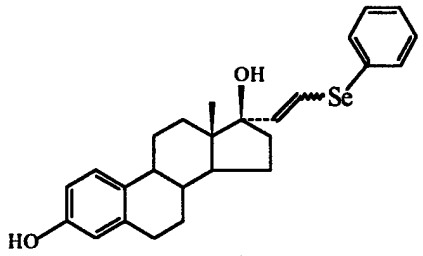


II

The synthesis of our target compounds to date had relied on traditional solution phase chemistry. In order to prepare new derivatives containing a variety of functional groups or existing as positional isomers, we considered approaches that could generate a large number of compounds more easily. The logical choice was solid phase synthesis. We envisioned that we could append our steroid to the inert polymer support, divide it into discrete aliquots, perform the requisite synthetic transformation, remove its individual products from the support and then characterize them. While a significant body of literature existed for solid phase synthesis (SPS) with steroids[4–9] and for Stille coupling,[10–12] there were no prior reports on the specific application that we wished to carry out. For example, Poirer et al., has described solid phase transformations of both androstanes and 16α-substituted estradiols,[4] however, neither employed transformations comparable to those we would require. Similarly, several groups have reported the use of the Stille reaction to couple aromatic and alkyl groups[10–12] but with fewer structural constraints than those imposed by the estrogen scaffold. Therefore, this work involved developing new methods to achieve our objectives.

*Keywords*: solid phase synthesis; estrogen receptor probes; carboxylation; hydrostannylation; Stille reaction.

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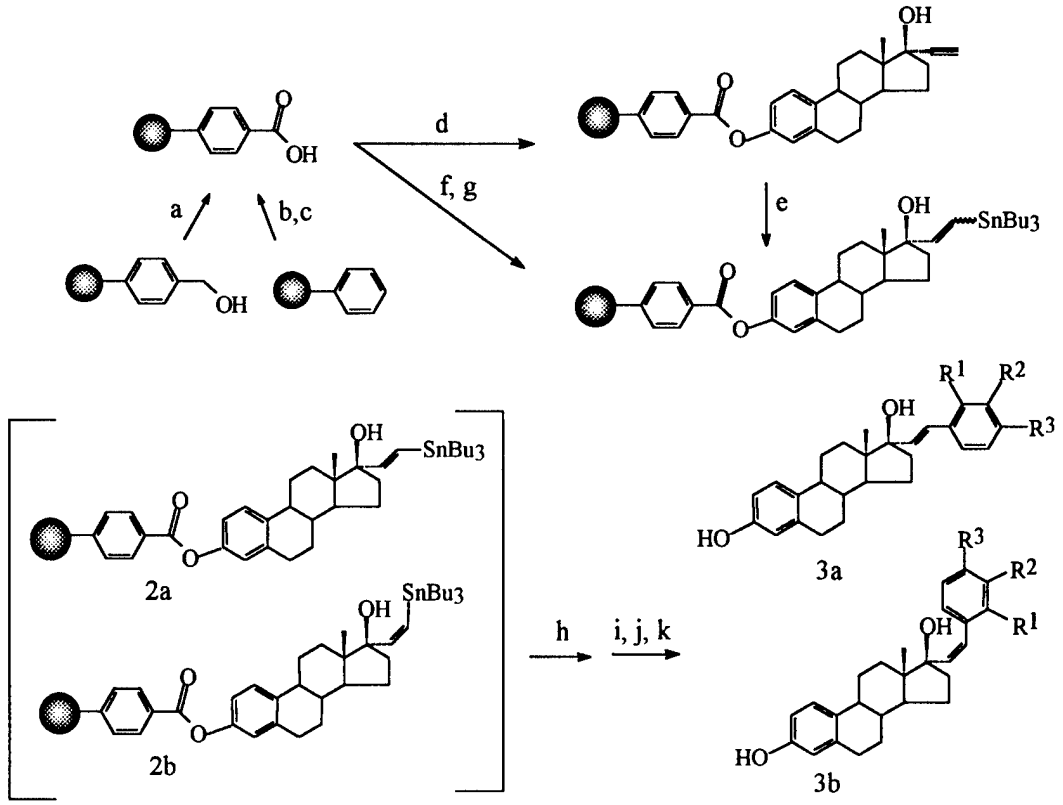



**Scheme 1.** Reagent: (a) Jones reagent ($H_2Cr_2O_4$, $H_2SO_4$, acetone); (b) *n*-BuLi, TMEDA, cyclohexane, 50°C; (c) Dry ice, THF; (d) 17α-Ethynyl estradiol, DCC, DMAP, $CH_2Cl_2$; (e) $HSnBu_3$, $Et_3B$, THF, 50–60°C; (f) 17α-Ethynyl estradiol, $HSnBu_3$, $Et_3B$, THF, 50–60°C; (g) DCC, DMAP, $CH_2Cl_2$; (h) R-Aryl-X, Pd $(PPh_3)_4$, BHT, toluene, $N_2$, reflux; (i) 5 N-NaOH in $CH_3OH$–Dioxane (1:3); (j) 5%-$CH_3COOH$; (k) 10%-$NaHCO_3$.

In this report we demonstrate our approach to developing the solid phase synthesis of the 17α-substituted phenylvinyl estradiols. This involved coupling the steroid intermediates to the resin, identifying appropriate reaction conditions and cleaving the final products from the resin. The result is a reliable method for generating a novel series of functionalized estradiols which can be evaluated for their biological properties.

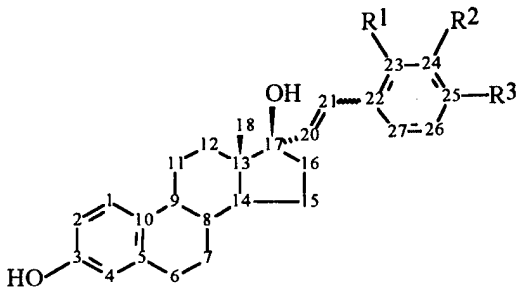
The approach that we selected incorporated several features. First, we chose the carboxylated resins because the estrogen could be selectively coupled through its phenolic linkage to the polymer and the ultimate cleavage of the ester bond at the end of the synthetic sequence would pose few problems. Use of an ether linkage would require either acidic or reductive cleavage, which would not be compatible with the functional groups present in the intermediates or final products. Similarly, amides, carbamates and photolabile links could also present potential problems at various steps of the process. Esterification at the 3-position, however, would not interfere with either the hydrostannylation or the palladium (0) catalyzed coupling reactions that would occur at the 17α-position. The integrity of the tertiary alcohols, *E/Z*-styryl groups, or functionality on the terminal phenyl group would be compromised if conditions other than a mild base were used to remove the product from the resin.

## Results and Discussion

One of the key elements of the synthetic scheme was the selection of a linker that could be both formed and cleaved under mild conditions. This was based on our observations that 17α-substituted estradiols were unstable under strongly acidic conditions such as those frequently used to release products from the resins. Therefore our resin of choice was carboxylated polystyrene which could be esterified under neutral conditions and ultimately cleaved with mild base. Our first example (compound **8a**) was prepared using the carboxylated resin obtained either by oxidation of a Wang resin using Jones reagent[13] or by carboxylation of a polystyrene resin via lithiation with *n*-butyl lithium.[14] The reactions for both methods were easily monitored by the appearance of the 1700 $cm^{-1}$ absorption in the FT-IR spectrum. The loading capacity of our carboxylated resins was determined by coupling 17α-ethynyl estradiol onto the resins using DCC in the presence of catalytic amount of DMAP and measuring its subsequently cleaved estradiol derivatives from the aliquot of the resins. The loading[15] of oxidized Wang resin was 0.4–0.6 mmol $g^{-1}$ and that of carboxylated polystyrene was 1.5–1.9 mmol $g^{-1}$. Once we confirmed the utility of coupling through the ester linkage using carboxy polystyrene resin we employed the commercially available carboxy polystyrene for the remainder of our studies. The loading yield of the reaction using the resins with already known loading capacity (2.47 mmol $g^{-1}$) was 82%. The yield was determined by 'cleave and characterize' methods.

Synthesis of the analogs (Scheme 1) commenced by coupling the 3-phenolic group of 17α-ethynyl estradiol to the carboxy polystyrene resin. An antimony (III) chloride

**Table 1.** Yields (%) of Stille coupling reaction using solid phase synthesis



| Compound | $R^1$ (*ortho*) | $R^2$ (*meta*) | $R^3$ (*para*) | Yield (%) |
|---|---|---|---|---|
| **4a**:*E* | $CF_3$ | H | H | 38 |
| **5a**:*E* | H | $CF_3$ | H | 33 |
| **6a**:*E* | H | H | $CF_3$ | 49 |
| **6b**:*Z* | H | H | $CF_3$ | 17 |
| **7a**:*E* | $CH_3$ | H | H | 38 |
| **8a**:*E* | H | $CH_3$ | H | 75 |
| **8b**:*Z* | H | $CH_3$ | H | 54 |
| **9a**:*E* | H | H | $OCH_3$ | 36 |

assay confirmed the presence of the steroids on the resins.[16–18] The absence of color change with bromocresol green suggested that no free carboxylic acid groups remained on the resin.[19] The appearance of a peak at 3301 $cm^{-1}$ in the IR spectrum, corresponding to the C–H stretch of the ethynyl group, also confirmed the reaction and a shift of carbonyl absorption to higher frequency (from 1690 to 1734 $cm^{-1}$) was also observed.

The subsequent hydrostannylation step incorporated either the use of hydrostannylation of bound ethynyl estradiol (Method A) or hydrostannylation of ethynyl estradiol in solution phase synthesis followed by coupling to the resin (Method B). The resin-bound 17α-ethynyl estradiol was hydrostannylated with tributyltin hydride using triethylborane as a radical initiator[20] to afford a mixture of the 17α-*E*/*Z*-tri-*n*-butylstannylvinyl estradiol in 20–30% (0.12 mmol $g^{-1}$ of *E*, 0.01 mmol $g^{-1}$ of *Z*) loading yields. Varying the reaction conditions, e.g. different solvents, temperatures, or reaction times, did not improve the yields. Therefore, a direct coupling of 17α-*E*/*Z*-tri-*n*-butylstannylvinyl estradiols used to overcome the low efficiency of this step. 17α-Ethynyl estradiol was hydrostannylated at 60°C and the crude mixture was directly transferred to the resin slurry in $CH_2Cl_2$. The mixture was treated with a 2–3 fold excess of DCC and a catalytic amount of DMAP was added. The loading yield for the coupling reaction was 0.59 mmol $g^{-1}$ with a *Z*/*E* ratio=1:20. The low loading yield was due to use of the acetic acid for the protonation of phenoxide ion after cleavage, subjecting the products to protiodestannylation and reducing the expected loading yield. Because the cleavage after hydrostannylation did not provide a precise loading yield, we subsequently used the dry weight difference between pre- and post-reaction to determine the loading yield. Using the dry weight difference method, the yield for the hydrostannylation reaction was 1.55 mmol $g^{-1}$ for both *E*- and *Z*-isomers. Because hydrostannylation on the resin did not afford satisfactory yields, Method B was the method of choice. As we have previously reported[21] the ratio of *E* and *Z* isomers is a function of the reaction temperature, time and stoichiometric ratio of tributyltin hydride to alkyne. At 60°C the reaction generated greater than 20:1 (*E*/*Z*) ratio bound to the solid phase. To increase the ratio of the *Z*-isomer, triethylborane was used as a radical initiator and the reaction was run at low temperature. The proportion of the *Z*-isomer (*Z*/*E*=1:10) increased, however, the reaction required a longer time and the loading yield for the hydrostannylation was slightly lower than at higher temperature (1.44 mmol $g^{-1}$ by the dry weight difference method) because of more unreacted 17α-ethynyl estradiol in the reaction mixture.

The resin-bound hydrostannnylated estradiol was subjected to the Stille coupling reaction[22] using a variety of substituted aryl halides to generate the target compounds (Table 1). As shown in Scheme 1, $Pd(PPh_3)_4$ was used as the catalyst for the reaction and 3,5-di-*t*-butyl-4-hydroxytoluene (BHT) was added as a scavenger. The use of $Pd(PPh_3)_4$ generated an insoluble by-product that caused coloration of the resin, however, it was easily removed by rinsing it through the built-in filter (50–70 μm). After completion of all the reaction steps, the product was cleaved from the resin by saponification with 5 N NaOH dissolved in $CH_3OH$–Dioxane (1:3).

As shown in Table 1, the unoptimized yields of the Stille reactions on solid phase ranged from 17–75%, comparable to those observed for solution phase synthesis.[23] Compounds **5a** (*para*-trifluoromethylphenyl, *E*-isomer) and **5b** (*para*-trifluoromethylphenyl, *Z*-isomer) were isolated from the Stille reaction in a ratio of 98:2. Compound **7a** (*meta*-methylphenyl, *E*-isomer) and **7b** (*meta*-methylphenyl, *Z*-isomer) were also obtained in a ratio of 96:4. Although the *Z*-tri-*n*-butylstannyl vinyl estradiol was initially present on the resin, no *Z*-isomers of compound **3a**, **4a**, **6a** or **8a** were isolated from the Stille coupling, instead, 17α-vinyl estradiol, resulting from protiodestannylation was recovered as a side product. Because an excess of reagent was used to drive the reaction to completion, unreacted hydrostannylated 17α-*E*/*Z*-(tri-*n*-butylstannyl)-vinyl estradiol was not detected after the Stille reaction. It is possible that the *Z*-isomers either isomerized to thermodynamically more stable *E*-isomers under the conditions required for the Stille reaction or underwent protiodestannylation. As previously observed, the *Z*-isomer is much more susceptible to protiodestannylation than the *E*-isomer and the appearance of the side product under either solid phase or solution phase synthesis was approximately the same.

The isolated product were characterized by standard spectroscopic methods (FT-IR, $^1H$ and $^{13}C$ NMR) and analytical methods. The data were consistent with the proposed structures. Stereochemical assignments for compounds **5a** and **5b** were based on the $C_{20}$, $C_{21}$ olefinic proton coupling constants for which *E*=16 Hz and *Z*=12.9 Hz, respectively. For compounds **7a** and **7b**, the observed coupling constants were 18.2 Hz for the $C_{20}$ *E*-vinyl proton and 13.1 Hz for the $C_{20}$ *Z*-vinyl proton. In $^{13}C$ NMR, long range couplings were observed for the compounds **3a**–**5a** and **5b** containing the trifluoromethyl group. Coupling with strongly electronegative fluorine was found at the carbon directly attached to the fluorine ($^1J_{C-F}$) and one ($^2J_{C-F}$) and two carbons distant ($^3J_{C-F}$). The carbons appeared as quartets and the coupling constants

were approximately $^{1}J_{C-F}$=270 Hz, $^{2}J_{C-F}$=32 Hz, $^{3}J_{C-F}$ =3–5 Hz, respectively.

Initial biological evaluation of these compounds indicates that they retain substantial affinity for the ER-LBD (results to be published elsewhere). Because both the properties of the aryl substitutent and its position on the ring (*o/m/p*) appear to affect the receptor binding, a more extensive evaluation of the derivatives is required.

In conclusion, we have applied the Solid Phase Synthesis methodology using carboxylated resins to generate a series of novel ER-LBD ligands. The initial objectives of this study, the simplification of the purification steps and the simultaneous production of both *E*- and *Z*-isomers, were largely achieved. The products were in acceptable yields, however no attempt had been made at this point to optimize conditions and clearly the yields could be improved. Analysis of the products indicated that the initial method provided only the *E*-isomer for most of the target compounds even though both *E* and *Z*-isomers were present after hydrostannylation reaction. We anticipate that modifications in both the coupling and cleavage steps would improve the yields for the chemically more sensitive *Z*-isomers. Nevertheless, this study has demonstrated the feasibility of solid phase synthesis for generating a variety of functionalized estradiol derivatives. Based on our preliminary biological results, we anticipate that further modifications of the phenyl group will yield promising results and we intend to adapt these methods for use in a combinatorial approach to generate diverse target compounds as ER-LBD ligands.

## Experimental

### Materials

Reagents and solvents were obtained from commercial sources (Aldrich and Sigma) and were used without further purification. Wang resins and carboxylated polystyrene resins were obtained from Novabiochem. The loading capacities of the resins, 0.75 mmol $g^{-1}$ for the Wang resin and 2.47 mmol $g^{-1}$ for the polystyrene resin, were determined by the manufacturer.

### General methods

A specially designed flask which had a glass frit, through which the reaction mixture could be filtered by applying pressure, was used for the solid phase synthesis. Purifications for the intermediates were done by rinsing resins three times with the following solvents: $CH_2Cl_2$, THF, DMF, MeOH, $CH_2Cl_2$. The cleaved products were purified on a silica gel column chromatography using the appropriate solvents and were characterized by melting point, NMR, IR and elemental analysis. Melting points were determined in open capillary on an Electrothermal Melting Point Apparatus and were uncorrected. IR spectra were recorded on a Perkin–Elmer Model 1600 FT-IR spectrometer. $^{1}H$ and $^{13}C$ NMR spectra were obtained with a Varian XL-300 NMR spectrometer at 300 MHz in $CDCl_3$, acetone-$d_6$, or DMSO-$d_6$ as a solvent. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). As on-resin reaction monitoring methods, color tests and FT-IR methods were used. Bomocresol green (0.5% in ethanol, pH=8) was used to assay for free carboxylic acids.[18] The color of the stock solution was dark blue and changed to yellow in the presence of free carboxy groups. Antimony (III) chloride solution (25% in $CCl_4$) was also used to determine whether the steroid (17α-ethynyl estradiol) was coupled to the resin and a positive test result for the presence of estradiol was indicated by the color purple.[16–18] In addition, a spectroscopic method (FT-IR) was facilitated to detect chromophore change by reaction.

### Preparation of the carboxylated resin

**(Method A).** The Wang resins (1 g, 0.75 mmol) were swelled in the $CH_2Cl_2$ overnight and rinsed twice with THF, $CH_3OH$, $CH_2Cl_2$ and acetone. Acetone (5 mL) was added to the swelled resins. To the slurry was added 1 mL of Jones reagent[13] in a dropwise manner. The mixture was allowed to stand at room temperature for 24 h. The resin mixture was rinsed twice with water–acetone (1:1), $CH_3OH$, DMF, DMSO and $CH_2Cl_2$ and dried in vacuo. The loading capacity after the carboxylation reaction was 0.4–0.6 mmol $g^{-1}$, which was determined with the coupling of 17α-ethynyl estradiol to the resin. The aliquot of the resins was characterized by FT-IR. FT-IR (KBr) $\nu$: 3000–3500 (OH, broad), 1690 (C=O, broad), 1603, 1492, 1452 (aromatic ring), 1279 (C–O).

**(Method B).** The carboxylation of a polystyrene resin was accomplished using the method described by Farrall et al.[14] FT-IR (KBr) $\nu$: 3420 (OH, broad), 1630 (C=O, broad), 1200–1400 (C–O, broad). Loading capacity: 1.5–1.9 mmol $g^{-1}$.

### Coupling 17α-ethynyl estradiol to the resins

The carboxylated Wang resin (2.3 g) or polystyrene resin (2.5 g) was placed in the reactor equipped with a magnetic stirrer. The resin was swelled in the $CH_2Cl_2$ for 5 h and washed sequentially with THF, DMF, $CH_3OH$, THF and $CH_2Cl_2$. To the resin was added 0.23 g (1.1 mmol) of dicyclohexylcarbodiimide (DCC) and 5 mL of $CH_2Cl_2$ and the mixture was mildly stirred for 10 min. To the slurry was added 0.75 g (2.6 mmol) of 17α-ethynyl estradiol dissolved in 10 mL of $CH_2Cl_2$–DMF (9:1) solvent and catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred for 5 min and then allowed to stand at room temperature for 24 h. The resin was washed three times with $CH_2Cl_2$, $CH_3OH$, IPA (60°C), THF and DMF (60°C).[24] The rinsed resin was dried under vacuum for 5 h. The actual loading of the resin was determined by quantitative measurement of the material by cleavage from known weight of resin using 5 N-NaOH in $CH_3OH$–dioxane (1:3). The resin-bound steroids were characterized by FT-IR and the cleaved compounds by $^{1}H$ and $^{13}C$ NMR before proceeding to the next step. The loading capacity of each resin was shown in Method A and B; FT-IR (KBr) $\nu$: 3437 (17β-OH), 3301 (17α-C≡C–H), 1735 (C=O), 1607, 1493, 1452 (aromatic ring), 1216(C–O).

## Hydrostannylation

**(Method A).** The 17α-ethynyl estradiol coupled to the resin (0.49 g, 0.57 mmol $g^{-1}$) was placed in a dry 25 mL reaction flask equipped with a reflux condenser and a magnetic stirrer and was swelled in THF for 1 h. To the slurry in the dry THF were treated triethylborane (0.7 mL) and tributyltin hydride (1 mL).[20] The mixture was allowed to stand at 60–70°C for 48 h under a nitrogen atmosphere. The reaction mixture was washed three times each with $CH_2Cl_2$, $CH_3OH$, DMF, $CH_2Cl_2$ and ethyl acetate and the resultant resin was dried in vacuo. An aliquot of the resins was cleaved with 5 N NaOH in $CH_3OH$–$CH_2Cl_2$ (1:2) to afford a mixture of *E*- and *Z*-isomers. The mixture was separated by chromatography on the silica gel to give a 23% (0.13 mmol $g^{-1}$) yield of products, consisting of 21% (0.12 mmol $g^{-1}$) of the *E*-isomer and 2% (0.01 mmol $g^{-1}$) of the *Z*-isomer. $R_f$ (*Z*-isomer)=0.58 (hexane–ethyl acetate, 4:1); $R_f$ (*E*-isomer)=0.44 (hexane–ethyl acetate, 4:1); Amorphous; $^1$H NMR ($CDCl_3$, 300 MHz, δ), 0.88 (s, 3H, $C_{18}$-methyl-H), 1.2–2.4 (m, steroid envelope and tributyl-stannyl-H), 2.7–2.9 (m, 2H, $C_6$-H), 6.06 (d, 1H, *J*=19.4 Hz, $C_{21}$ vinyl-H), 6.22 (d, 1H, *J*=19.4 Hz, $C_{20}$ vinyl-H), 6.79 (d, 1H, *J*=2.4 Hz, $C_4$-H), 6.84 (dd, 1H, *J*=2.6, 8.4 Hz, $C_2$-H), 7.28 (d, 1H, *J*=8.8 Hz, $C_1$-H); $^{13}$C NMR ($CDCl_3$), 9.6 ($C_{22}$, 4C), 13.7 ($C_{24}$, 4C), 14.2 ($C_{18}$), 23.4 ($C_{15}$), 26.4 ($C_{11}$), 27.3 ($C_{25}$, 4C), 27.4 ($C_7$), 29.2 ($C_{23}$, 4C), 29.6 ($C_6$), 32.4 ($C_{12}$), 35.9 ($C_{16}$), 39.4 ($C_8$), 43.8 ($C_9$), 46.7 ($C_{13}$), 49.0 ($C_{14}$), 85.6 ($C_{17}$), 112.6 ($C_2$), 115.2 ($C_4$), 124.6 ($C_{21}$), 126.5 ($C_1$), 132.7 ($C_{10}$), 138.3 ($C_5$), 152.4 ($C_{20}$), 153.3 ($C_3$); FT-IR (KBr) ν: 3445 (17β-OH, broad), 1719 (C=O), 1653 (C=C), 1607, 1493, 1451 (aromatic ring), 1217 (C–O).

**(Method B).** The 17α-ethynyl estradiol (3 g, 10 mmol) was dissolved in THF and treated with triethylborane (2 mL, 17 mmol) and tributyltin hydride (3 g, 11 mmol). The mixture was stirred with a magnetic stirrer at 60°C for 16 h. The crude mixture (7.73 g) was evaporated to dryness, redissolved in the $CH_2Cl_2$, and transferred to the swelled resin (5 g) in $CH_2Cl_2$ in the presence of DCC. A catalytic amount of DMAP was added to the mixture, which was allowed to stand for 24 h. The resultant functionalized resin was treated as previously described. The total loading for both *E*- and *Z*-isomers was 0.59 mmol $g^{-1}$ with 0.56 mmol $g^{-1}$ of *E*-isomer and 0.03 mmol $g^{-1}$ of *Z*-isomer, however, by the dry weight difference between pre- and post-reaction, the loading for both *E*- and *Z*-isomers was 1.55 mmol $g^{-1}$.

## Electrophilic destannylation on the resin

The Stille reaction was used to couple the anchored *E*- and *Z*-stannylvinyl estradiol to aryl halides. The resin was added to the reaction flask, swelled in the $CH_2Cl_2$, subsequently treated with 10 mL of anhydrous toluene. To the resultant slurry was added a 3–4 fold excess of the functionalized aryl halide, 1–2 crystals of 3,5-di-*t*-butyl-4-hydroxytoluene (BHT), and $Pd(PPh_3)_4$.[13–15] The reaction was allowed to proceed at 90–100°C for 24 h. After cooling, the resin was washed as previously described, dried in vacuo and weighed.

## Cleavage

The resin was swelled in $CH_2Cl_2$ (10 mL) containing 3 mL of 5 N-NaOH in $CH_3OH$–Dioxane (1:3), and stirred for 1 h. This cleavage step was repeated three times. Most of the product was collected from the first attempt, a small amount by second hydrolysis and almost none from the third trial. The fractions were combined, evaporated to dryness and partitioned between ethyl acetate and water. Acetic acid (1 mL, 5%) was added. The organic phase was washed with 10% aqueous $NaHCO_3$ to remove the residual acetic acid, dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by silica gel column chromatography or by recrystallization from the appropriate solvent.

**17α-20*E*-21-(2-Trifluoromethylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (17α-*E*-(2-trifluoro methylphenyl)-vinyl estradiol) (4a).** Yield=38%; $R_f$=0.19 (hexane–ethyl acetate, 4:1); mp 224–225°C; $^1$H NMR (300 MHz, Acetone-$d_6$, δ) 1.02 (s, 3H, $C_{18}$ methyl-H), 1.2–2.4 (m, steroid envelope), 2.7–2.9 (m, 2H, $C_6$-H), 3.98(s, 1H, 17β hydroxyl-H), 6.53 (d, 1H, *J*=2.3 Hz, $C_4$-H), 6.58 (dd, 1H, *J*=2.6, 8.5 Hz, $C_2$-H), 6.64 (d, 1H, *J*=15.7 Hz, $C_{20}$ vinyl-H), 7.0 (dd, 1H, *J*=2.5, 15.8 Hz, $C_{21}$ vinyl-H), 7.07 (d, 1H, *J*=8.7 Hz, $C_1$-H), 7.42 (t, 1H, *J*=7.8 Hz, $C_{26}$-H), 7.60 (t, 1H, *J*=7.3 Hz, $C_{25}$-H), 7.69 (d, 1H, *J*=7.8 Hz, $C_{27}$-H), 7.81 (d, 1H, *J*=8.3 Hz, $C_{24}$-H), 7.98 (s, $C_3$ hydroxy-H); $^{13}$C NMR (75.4 MHz, Acetone-$d_6$, δ) 14.7 ($C_{18}$), 24.1 ($C_{15}$), 27.2 ($C_{11}$), 28.3 ($C_7$), ($C_6$), 33.4 ($C_{12}$), 37.5 ($C_{16}$), 40.7 ($C_8$), 44.6 ($C_9$), 48.4 ($C_{13}$), 50.0 ($C_{14}$), 84.3 ($C_{17}$), 113.5 ($C_2$), 115.9 ($C_4$), 123.4 ($C_{21}$), 125.6 (q, *J*=273.2 Hz, $C_{28}$:$CF_3$), 126.4 (q, *J*=5.8 Hz, $C_{24}$), 127.0 ($C_1$), 127.4 (q, *J*=29.4 Hz, $C_{23}$), 127.8 ($C_{26}$), 128.6 ($C_{27}$), 132.0 ($C_{25}$), 133.2 ($C_{10}$), 137.9 ($C_{22}$), 139.1 ($C_5$), 142.4 ($C_{20}$), 155.9 ($C_3$); Anal. Calcd for $C_{27}H_{29}O_2F_3$: C, 73.30; H, 6.56. Found: C, 73.04; H, 6.68.

**17α-20*E*-21-(3-Trifluoromethylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (17α-*E*-(3-trifluoro methylphenyl)-vinyl estradiol) (5a).** Yield=33%; $R_f$ (*E*-isomer)=0.19 (hexane–ethyl acetate, 4:1); mp 244–246°C; $^1$H NMR (300 MHz, Acetone-$d_6$, δ), 1.01 (s, 3H, $C_{18}$-methyl), 1.2–2.4 (m, steroid envelope), 2.7–2.9 (m, 2H, $C_6$-H), 3.98 (s, 1H, 17β hydroxyl-H), 6.53 (d, 1H, *J*=2.6 Hz, $C_4$-H), 6.58 (dd, 1H, *J*=2.6, 8.3 Hz, $C_2$-H), 6.74 (d, 1H, *J*=16 Hz, $C_{21}$ vinyl-H), 6.84 (d, 1H, *J*=16 Hz, $C_{20}$ vinyl-H), 7.06 (d, 1H, *J*=8.3 Hz, $C_1$-H), 7.54–7.56 (m, 2H, $C_{25}$, $C_{27}$-H), 7.75–7.79 (m, 2H, $C_{23}$, $C_{26}$-H), 7.93 (s, $C_3$-hydroxy-H); $^{13}$C NMR (75.4 MHz, Acetone-$d_6$, δ), 14.7 ($C_{18}$), 24.1 ($C_{15}$), 27.3 ($C_{11}$), 28.3 ($C_7$), ($C_6$), 33.5 ($C_{12}$), 37.5 ($C_{16}$), 40.7 ($C_8$), 44.6 ($C_9$), 48.4 ($C_{13}$), 50.1 ($C_{14}$), 84.2 ($C_{17}$), 113.5 ($C_2$), 115.9 ($C_4$), 123.6 (q, *J*=5.6 Hz, $C_{25}$), 124.1 (q, *J*=3.7 Hz, $C_{23}$), 125.4 (q, *J*=271 Hz, $C_{28}$:$CF_3$), 126.0 ($C_{26}$), 127.0 ($C_1$), 130.2 ($C_{21}$), 130.7 ($C_{27}$), 131.2 (q, *J*=32 Hz, $C_{24}$), 132.0 ($C_{10}$), 138.4 ($C_5$), 139.7 ($C_{20}$), 139.9 ($C_{22}$), 155.9 ($C_3$); Anal. Calcd for $C_{27}H_{29}O_2F_3$: C, 73.30; H, 6.56. Found: C, 73.42; H, 6.68.

**17α-20*E*-21-(4-Trifluoromethylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (17α-*E*-(4-trifluoro methylphenyl)-vinyl estradiol) (6a).** Yield=49%; $R_f$= 0.15 (hexane–ethyl acetate, 4:1); mp 215–217°C; $^1$H

NMR (Acetone-$d_6$, 300 MHz, $\delta$), 1.02 (s, 3H, $C_{18}$ methyl-H), 1.2–2.4 (m, steroid envelope), 2.7–2.9 (m, 2H, $C_6$-H), 3.90 (s, 1H, 17β hydroxyl-H), 6.53 (d, 1H, $J$=2.6 Hz, $C_4$-H), 6.58 (dd, 1H, $J$=2.6, 8.4 Hz, $C_2$-H), 6.73 (d, 1H, $J$=16 Hz, $C_{21}$ vinyl-H), 6.85 (d, 1H, $J$=16 Hz, $C_{20}$ vinyl-H), 7.07 (d, 1H, $J$=8.3 Hz, $C_1$-H), 7.64 (d, 2H, $J$=8.7 Hz, $C_{23}$, $C_{27}$-H), 7.70 (d, 2H, $J$=8.6 Hz, $C_{24}$, $C_{26}$-H), 8.0 (s, $C_3$-hydroxy-H); $^{13}$C NMR (75.4 MHz, Acetone-$d_6$, $\delta$) 14.7 ($C_{18}$), 24.1 ($C_{15}$), 27.3 ($C_{11}$), 28.3 ($C_7$), ($C_6$), 33.5 ($C_{12}$), 37.6 ($C_{16}$), 40.7 ($C_8$), 44.6 ($C_9$), 48.5 ($C_{13}$), 50.2 ($C_{14}$), 84.2 ($C_{17}$), 113.5 ($C_2$), 115.9 ($C_4$), 125.4 (q, $J$=270.6 Hz, $C_{28}$:$CF_3$), 126.0 ($C_{21}$), 126.2 (q, $J$=3.5 Hz, $C_{26}$), 126.2 (q, $J$=3.5 Hz, $C_{24}$), 127.0 ($C_1$), 127.6 ($C_{23}$, $C_{27}$), 128.9 (q, $J$=32 Hz, $C_{25}$), 132.0 ($C_{10}$), 138.4 ($C_5$), 140.6 ($C_{20}$), 142.7 ($C_{22}$), 155.9 ($C_3$); Anal. Calcd for $C_{27}H_{29}O_2F_3$: C, 73.30; H, 6.56. Found: C, 73.36; H, 6.79.

**17α-20*Z*-21-(4-Trifluoromethylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (17α-*Z*-(4-trifluoro methylphenyl)-vinyl estradiol) (6b).** Yield=17%; $R_f$= 0.29 (hexane–ethyl acetate, 4:1); $^1$H NMR (300 MHz, Acetone-$d_6$, $\delta$) 0.97 (s, 3H, $C_{18}$ methyl-H), 1.2–2.4 (m, steroid envelope), 2.7–2.9 (m, 2H, $C_6$-H), 3.89 (s, 1H, 17β hydroxyl-H), 6.12 (d, 1H, $J$=12.9 Hz, $C_{21}$ vinyl-H), 6.48–6.62 (m, 3H, $C_2$, C $_4$, $C_{20}$ vinyl-H), 7.11 (d, 1H, $J$=8.1 Hz, $C_1$-H), 7.59 (d, 2H, $J$=8.4 Hz, $C_{23}$, $C_{27}$-H), 7.80 (d, 2H, $J$=8.4 Hz, $C_{24}$, $C_{26}$-H), 7.95 (s, $C_3$ hydroxy-H).

**17α-20*E*-21-(2-Methylphenyl)-19-norpregna-1,3,5(10), 20-tetraene-3,17β-diol (17α-*E*-(2-methylphenyl)-vinyl estradiol) (7a).** Yield=38%; $R_f$=0.18 (hexane–acetone, 4:1); mp 199–200°C; $^1$H NMR (Acetone-$d_6$, 300 MHz, $\delta$), 1.01 (s, 3H, $C_{18}$ methyl-H), 1.2–2.4 (steroid envelope), 2.34 (s, 3H, $C_{28}$ methyl-H), 2.7–2.9 (m, 2H, $C_6$-H), 3.84 (s, 1H, 17β hydroxyl-H), 6.44 (d, 1H, $J$=16 Hz, $C_{21}$ vinyl-H), 6.52–6.63 (m, 2H, $C_2$, $C_4$-H), 6.83 (d, 1H, $J$=16 Hz, $C_{20}$ vinyl-H), 7.07 (d, 1H, $J$=8.3 Hz, $C_1$-H), 7.10–7.15 (m, 3H, $C_{24}$, $C_{25}$, $C_{26}$-H), 7.48 (d, 1H, $J$=6.8 Hz, $C_{27}$-H), 7.97 (s, $C_3$ hydroxy-H); $^{13}$C NMR (75.4 MHz, Acetone-$d_6$, $\delta$) 14.7 ($C_{18}$), 19.9 ($C_{28}$: methyl), 24.1 ($C_{15}$), 27.3 ($C_{11}$), 28.3 ($C_7$), ($C_6$), 33.5 ($C_{12}$), 37.5 ($C_{16}$), 40.7 ($C_8$), 44.7 ($C_9$), 48.2 ($C_{13}$), 50.1 ($C_{14}$), 84.2 ($C_{17}$), 113.5 ($C_2$), 115.9 ($C_4$), 125.4($C_{26}$), 126.5 ($C_{25}$), 126.9 ($C_{24}$), 127.0 ($C_1$), 127.7 ($C_{21}$), 130.8 ($C_{27}$), 132.0 ($C_{10}$), 135.9 ($C_{20}$), 137.9 ($C_{22}$), 138.4 ($C_5$), 138.8 ($C_{23}$), 155.9 ($C_3$); Anal. Calcd for $C_{27}H_{32}O_2$: C, 83.51; H, 8.25. Found: C, 83.79; H, 8.65.

**17α-20*E*-21-(3-Methylphenyl)-19-norpregna-1,3,5(10), 20-tetraene-3,17β-diol (17α-*E*-(3-methylphenyl)-vinyl estradiol) (8a).** Yield=75%; $R_f$=0.17 (hexane–acetone, 4:1); mp 204–205°C; $^1$H NMR (300 MHz, Acetone-$d_6$, $\delta$), 1.00 (s, 3H, $C_{18}$ methyl-H), 1.2–2.4 (m, steroid envelope), 2.31 (s, 3H, $C_{28}$ methyl-H), 2.7–2.9 (m, 2H, $C_6$-H), 3.74 (s, 1H, 17β hydroxyl-H), 6.52–6.63 (m, 4H, $C_4$, $C_2$, $C_{21}$ vinyl, $C_{20}$ vinyl-H), 7.03 (d, 1H, $J$=7.3 Hz, $C_{25}$-H), 7.07 (d, 1H, $J$=8.7 Hz, $C_1$-H), 7.16–7.31 (m, 3H, $J$=7.4 Hz, $C_{23}$, $C_{26}$, $C_{27}$-H), 7.93 (s, 1H, $C_3$ hydroxy-H); $^{13}$C NMR (75.4 MHz, Acetone-$d_6$, $\delta$) 14.8 ($C_{18}$), 21.4 ($C_{28}$: methyl), 24.1 ($C_{15}$), 27.3 ($C_{11}$), 28.4 ($C_7$), ($C_6$), 33.5 ($C_{12}$), 37.4 ($C_{16}$), 40.8 ($C_8$), 44.7 ($C_9$), 48.3 ($C_{13}$), 50.2 ($C_{14}$), 84.2 ($C_{17}$), 113.6 ($C_2$), 116.0 ($C_4$), 124.4 ($C_{27}$), 127.0 ($C_1$), 127.7 ($C_{25}$), 127.8 ($C_{26}$), 128.5 ($C_{21}$), 129.2 ($C_{23}$), 132.2 ($C_{10}$), 137.0 ($C_{20}$), 138.4 ($C_5$), 138.7 ($C_{22}$, $C_{24}$), 155.9 ($C_3$); Anal. Calcd for $C_{27}H_{32}O_2$: C, 83.51; H, 8.25. Found: C, 83.23; H, 8.42.

**17α-20*Z*-21-(3-Methylphenyl)-19-norpregna-1,3,5(10), 20-tetraene-3,17β-diol (17α-*Z*-(3-methylphenyl)-vinyl estradiol) (8b).** Yield=54% (0.01 g); $R_f$=0.25 (hexane–acetone, 4:1); $^1$H NMR (300 MHz, Acetone-$d_6$, $\delta$) 0.95 (s, 3H, $C_{18}$ methyl-H), 1.2–2.4 (m, steroid envelope), 2.31 (s, 3H, $C_{28}$ methyl-H), 2.7–2.9 (m, 2H, $C_6$-H), 3.27 (s, 1H, 17β hydroxyl-H), 5.96 (d, 1H, $J$=13.1 Hz, $C_{21}$ vinyl-H), 6.44 (d, 1H, $J$=13.1 Hz, $C_{20}$ vinyl-H), 6.53 (d, 1H, $J$=2.6 Hz, $C_4$-H), 6.60 (dd, 1H, $J$=2.6, 8.3 Hz, $C_2$-H), 7.03 (d, 1H, $J$=7.3 Hz, $C_{25}$-H), 7.11 (d, 1H, $J$=8.3 Hz, $C_1$-H), 7.17 (t, 1H, $J$=7.6 Hz, $C_{26}$-H), 7.38–7.43 (m, 2H, $C_{23}$, $C_{27}$-H), 7.95 (s, 1H, $C_3$ hydroxy-H); $^{13}$C NMR (75.4 MHz, Acetone-$d_6$, $\delta$) 14.58 ($C_{18}$), 21.42 ($C_{28}$:methyl), 23.85 ($C_{15}$), 27.40 ($C_{11}$), 28.30 ($C_7$), ($C_6$), 32.97 ($C_{12}$), 38.4 ($C_{16}$), 40.9 ($C_8$), 44.7 ($C_9$), 48.8 ($C_{13}$), 50.1 ($C_{14}$), 84.3 ($C_{17}$), 113.6 ($C_2$), 116.0 ($C_4$), 127.1 ($C_1$), 127.8 ($C_{27}$), 128.1 ($C_{25}$), 128.3 ($C_{26}$), 129.7 ($C_{21}$), 131.4 ($C_{23}$), 132.0 ($C_{10}$), 137.1 ($C_{20}$), 137.6 ($C_{24}$), 138.45 ($C_5$) 138.5 ($C_{22}$), 155.9 ($C_3$); Anal. Calcd for $C_{29}H_{36}O_3$: C, 80.55; H, 8.33. Found: C, 80.00; H, 8.41.

**17α-20*E*-21-(4-Methoxyphenyl)-19-norpregna-1,3,5,(10), 20-tetraene-3,17β-diol (17α-*E*-(4-methoxyphenyl)-vinyl estradiol) (9a).** Yield=36%; $R_f$=0.23 ($CHCl_3$–$CH_3OH$, 99:1); $^1$H NMR (300 MHz, Acetone-$d_6$, $\delta$) 0.99 (s, 3H, $C_{18}$ methyl-H), 3.68 (s, 1H, 17β hydroxy-H), 3.78 (s, 3H, $C_{28}$:methoxy-H), 6.46 (d, 1H, $J$=16.1 Hz, $C_{21}$-H), 6.51–6.59 (m, 3H, $C_2$, $C_4$, $C_{20}$-H), 6.88 (d, 2H, $J$=8.8 Hz, $C_{24}$, $C_{26}$-H); 7.07 (d, 1H, $J$=8.3 Hz, $C_1$-H); 7.39 (d, 2H, $J$=8.8 Hz, $C_{23}$, $C_{27}$-H), 7.95 (s, 1H, $C_3$ hydroxy-H); $^{13}$C NMR (75.4 MHz, Acetone-$d_6$, $\delta$) 14.7 ($C_{18}$), 24.1 ($C_{15}$), 27.3 ($C_{11}$), 28.3 ($C_7$), ($C_6$), 33.4 ($C_{12}$), 37.3 ($C_{16}$), 40.7 ($C_8$), 44.7 ($C_9$), 48.2 ($C_{13}$), 50.0 ($C_{14}$), 55.5 ($C_{28}$:methoxy), 84.1 ($C_{17}$), 113.5 ($C_2$), 114.7 ($C_{24}$, $C_{26}$), 115.9 ($C_4$), 127.0 ($C_1$), 127.0 ($C_{21}$), 128.3 ($C_{23}$, $C_{27}$), 131.4 ($C_{22}$), 132.1 ($C_{10}$), 134.9 ($C_{20}$), 138.4 ($C_5$), 155.9 ($C_3$), 159.9 ($C_{25}$).

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# Conformational Studies on (17$\alpha$,20*Z*)-21-(*X*-Phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17$\beta$-diols Using 1D and 2D NMR Spectroscopy and GIAO Calculations of $^{13}$C Shieldings

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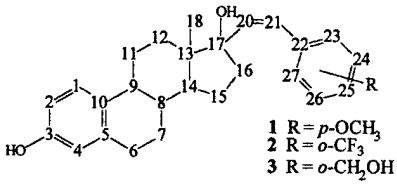


**Figure 1.** Structures of **1**–**3**.

RBA of 23 in vitro. In stark contrast, (17α,20*Z*)-(*o*-hydroxymethylphenyl)vinyl estradiol (**3**) exhibited significant agonist responses with an RBA of 140, giving **3** more potent estrogen binding affinity than estradiol itself.

Previous studies reveal a considerable interest in the conformation of steroids.[7] These studies indicated that the biological activity of these compounds was related to their conformation. Since the placement of a substituent in the *ortho* or *para* positions could affect the conformation and since the conformational characteristics of 17α-phenylvinyl steroids had not been studied previously, we undertook an investigation of the solution conformation of **1**–**3**. Understanding the preferred conformations is one aspect of an effort to correlate the distinctive biological responses derived from these new probes with their structures and ultimately to associate the responses with the ligand–receptor interactions.

The key conformational feature to establish for **1**–**3** is the positioning of the 17α side chain relative to the steroid skeleton. The conformation of the relatively rigid steroidal skeleton has been established previously by NMR and other methods.[8] In this study, we use molecular mechanics calculations to generate a set of possible conformations. Two types of NMR data are used in conjunction with the predicted conformations to evaluate which conformations are populated in solution. One approach is to use $^{13}C$ chemical shifts in a comparison with shifts predicted for each of the geometries generated from the molecular mechanics calculations. The predicted $^{13}C$ shifts come from empirically scaled GIAO (gauge including atomic orbitals) shielding calculations. The other approach is to compare $^{1}H-^{1}H$ nuclear Overhauser effects established in one- and two-dimensional experiments, 1D and 2D NOESY, with predicted interatomic distances.

**NMR Assignments.** Before NMR data could be used to evaluate the conformations of **1**–**3**, accurate $^{1}H$ and $^{13}C$ chemical shift assignments were required. The one-dimensional $^{1}H$ spectra of **1**–**3** in acetone-$d_6$ (Figures 2a, 3a, and 4a) reveal that even at 500 MHz, the low-frequency spectral regions (1.2–2.5 ppm) are unassignable directly as a result of the numerous overlapping signals of the 13 protons resonating in this region. In seeking further separation of the low-frequency region, other deuterated solvents were used, namely, benzene, benzene/acetone, chloroform, chloroform/acetone, and methylene chloride, but pure acetone provides the best separation. Resonances in the low-frequency region that could be readily assigned were the 6α,6β benzylic protons near 2.8 ppm and the C18 methyl $^{1}H$ signal at 0.9 ppm.[9] Prior literature reports on $^{1}H$ NMR assignments of estradiol and other steroids are in disagreement and were of little assistance in assigning the remaining low-frequency region.[10] No publication of $^{1}H$ spectral assignments for any 17α -vinyl-substituted estradiols exists.

The most efficient route to $^{1}H$ signal assignment was to first assign the $^{13}C$ spectrum. For **1**–**3**, the $^{13}C$ experimental shift assignments were based on the study by Dionne and Poirier on $^{13}C$ assignments of 17α-substituted estradiols and our own DEPT and HMBC experiments.[11] The $^{13}C$ shift assignments were further supported by theoretical shielding calculations (see below). A heteronuclear multiple quantum coherence (HMQC) experiment was performed to correlate proton signals with directly attached carbons. Because the $^{1}H$ chemical shift assignments derived from the HMQC experiment depended on the accuracy of the $^{13}C$ chemical shift assignments, other 2D experiments were performed to provide independent evidence. Homonuclear correlation spectroscopy (H,H-COSY) experiments were performed to correlate the assigned $^{1}H$ connectivities. The COSY cross-peaks confirmed the initial assignments made by the HMQC experiment. Starting with the unambiguous benzylic H6 signal at 2.8 ppm, the $^{1}H$ assignments of the entire aliphatic regions of **1**–**3** were confirmed.

The HMQC and H,H-COSY experiments clearly indicated the sites of attachment of all of the protons but did not distinguish between the α and β position of the methylene protons. This distinction was readily achieved by using 2D and 1D nuclear Overhauser effect spectroscopy (NOESY) experiments and by comparing coupling constants. Inspection of the $^{1}H$ NMR spectrum allows the axial protons, 7α and 6β, to be identified by their larger vicinal coupling constants. The equatorial proton, 11α, is assigned to the isolated signal around 2.4 ppm on the basis of its small coupling constants. The remaining β protons were assigned by the determination of transient NOEs using a 1D NOESY experiment, the 1D analogue of the 2D NOESY experiment.[12] The 1D NOESY experiment avoided problems associated with imperfect subtraction in NOE difference experiments.[13]

Using a selective Gaussian pulse, irradiation of the C18 methyl peaks of **1**–**3** gave signal enhancements for the β-protons at positions 8, 11, 12, 15, and 16 (Figures 2b, 3b, and 4b). These experiments were crucial in making chemical shift assignments, since they resolved β protons from overlapping regions containing α protons. For example, the spectrum of **2** shows a set of four overlapping protons at δ 1.65–1.8 for 12α, 12β, H14 and 15α.

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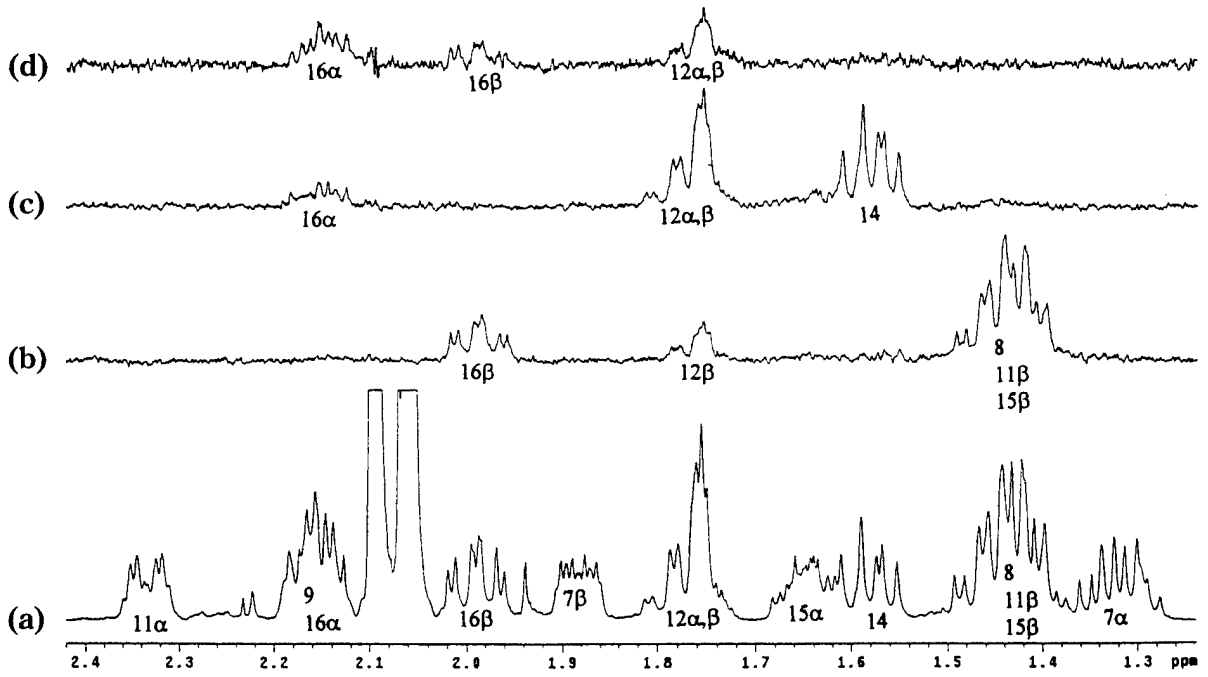


**Figure 2.** (a) Low-frequency spectral region of the 500 MHz $^1$H NMR spectra of **1** in acetone-$d_6$. Equivalent spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **1** obtained by selective irradiation of the C18 methyl (b), H20 (c), and H23/27 (d) using a Gaussian pulse. Spectra b and c are 5× the vertical scale of a. Spectra d is 10× the vertical scale of a.

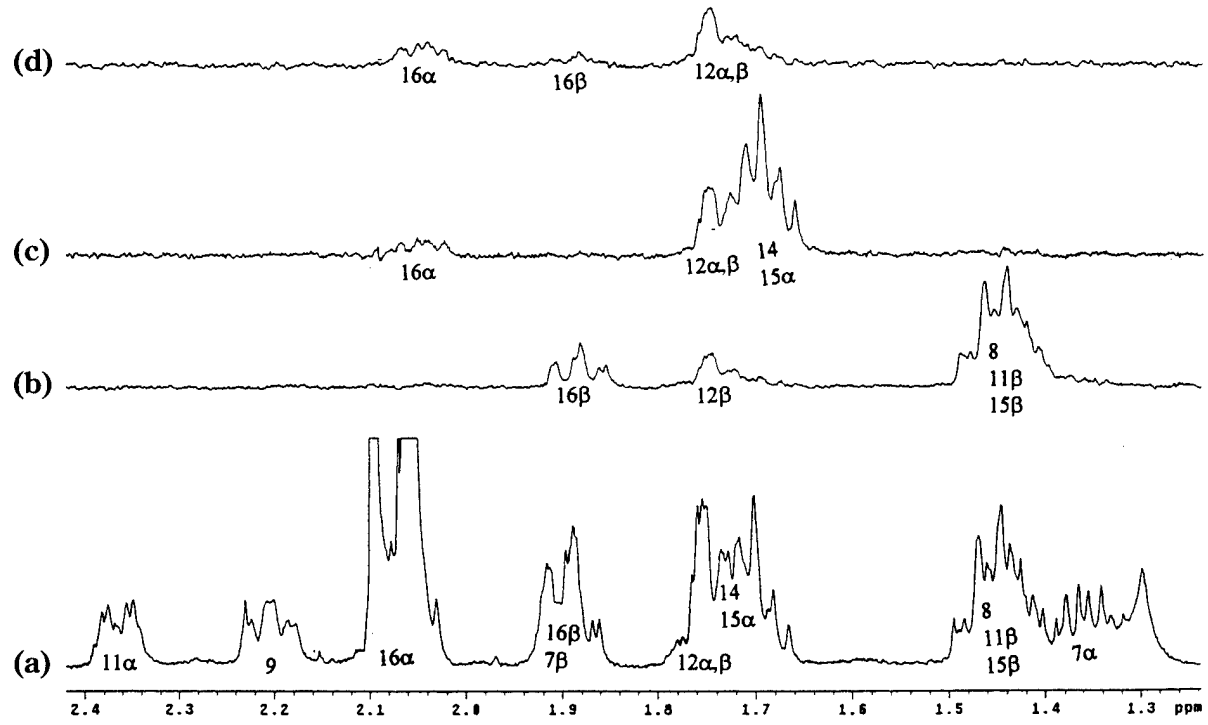


**Figure 3.** (a) Low-frequency spectral region of the 500 MHz $^1$H NMR spectra of **2** in acetone-$d_6$. Equivalent spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **2** obtained by selective irradiation of the C18 methyl (b), H20 (c), and H27 (d) using a Gaussian pulse. Spectra b and c are 5× the vertical scale of a. Spectra d is 10× the vertical scale of a.

Irradiation of the C18 methyl, in the 1D NOESY experiment, reveals at 1.75 ppm the expected 12$\beta$ signal from the overlapping region. The remaining assignments in this set are based on the HMQC of steroid **2** that shows that the H14 and 15α protons are slightly further upfield (1.7 and 1.72 ppm) than 12α or 12$\beta$. The remaining signal at 1.77 ppm can therefore be assigned to 12α. Assignments in the B and C ring were validated by other 1D NOESY experiments, including the irradiation of H1 that results in the expected enhancement of 11α and the irradiation of H6, yielding the expected 7α, 7$\beta$, and H8 enhancements. In summary, consideration of all the independent NMR experiments allowed the unambiguous assignment of all $^1$H and $^{13}$C resonances. Table 1 summarizes all of the $^1$H and $^{13}$C chemical shifts for **1**–**3**.

**Theoretical Carbon Chemical Shifts and Solution Conformations.** The predicted low-energy conformers of **1**–**3** were generated using the MM3 force field and

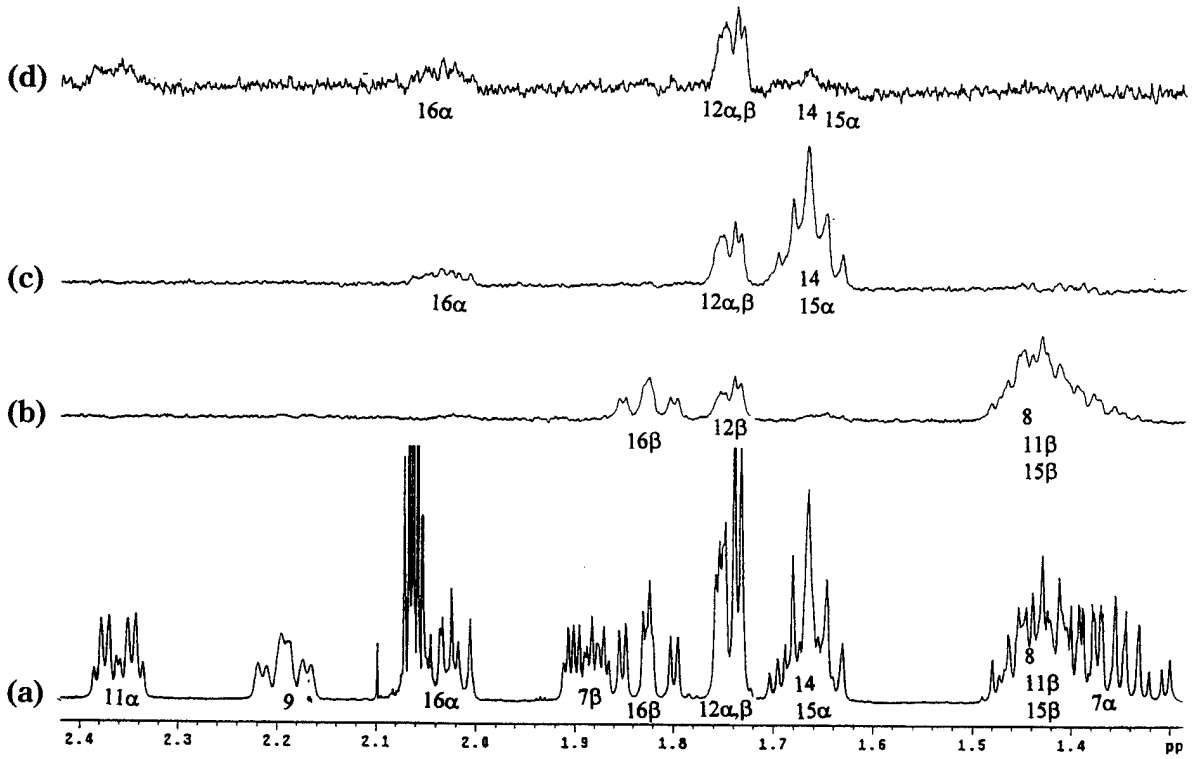


**Figure 4.** (a) Low-frequency spectral region of the 500 MHz $^1$H NMR spectra of **3** in acetone-$d_6$. Equivalent spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **3** obtained by selective irradiation of the C18 methyl (b), H20 (c), and H23/27 (d) using a Gaussian pulse. Spectra b and c are 5× the vertical scale of a. Spectra d is 15× the vertical scale of a.

**Table 1. $^1$H and $^{13}$C Chemical Shifts for 1–3**

| $^1$H | 1 | 2 | 3 | $^{13}$C | 1 | 2 | 3 |
|---|---|---|---|---|---|---|---|
| 1 | 7.12 | 7.12 | 7.12 | 1 | 126.9 | 127.4 | 126.5 |
| 2 | 6.60 | 6.62 | 6.62 | 2 | 113.5 | 113.9 | 113.2 |
| 4 | 6.54 | 6.60 | 6.58 | 3 | 155.8 | 155.0 | 153.2 |
| 6α | 2.75 | 2.78 | 2.79 | 4 | 115.8 | 116.2 | 115.2 |
| 6β | 2.80 | 2.81 | 2.82 | 5 | 138.3 | 139.1 | 137.5 |
| 7α | 1.32 | 1.38 | 1.34 | 6 | 29.9 | 30.7 | 29.8 |
| 7β | 1.88 | 1.88 | 1.88 | 7 | 28.5 | 28.7 | 27.9 |
| 8 | 1.43 | 1.48 | 1.43 | 8 | 40.7 | 41.2 | 40.2 |
| 9 | 2.18 | 2.20 | 2.18 | 9 | 44.5 | 45.0 | 44.0 |
| 11α | 2.33 | 2.38 | 2.36 | 10 | 131.9 | 131.9 | 131.9 |
| 11β | 1.46 | 1.45 | 1.46 | 11 | 27.3 | 27.7 | 26.8 |
| 12α | 1.77 | 1.77 | 1.76 | 12 | 32.6 | 33.7 | 33.0 |
| 12β | 1.75 | 1.75 | 1.74 | 13 | 48.7 | 49.0 | 48.0 |
| 14 | 1.57 | 1.70 | 1.66 | 14 | 49.9 | 50.8 | 49.9 |
| 15α | 1.64 | 1.72 | 1.68 | 15 | 23.7 | 24.4 | 23.4 |
| 15β | 1.41 | 1.43 | 1.40 | 16 | 38.3 | 39.3 | 38.4 |
| 16α | 2.16 | 2.06 | 2.02 | 17 | 83.8 | 85.8 | 84.8 |
| 16β | 1.98 | 1.90 | 1.82 | 18 | 14.5 | 14.8 | 14.6 |
| $CH_3$ | 0.96 | 0.90 | 0.88 | 20 | 135.1 | 138.7 | 138.0 |
| 20 | 5.88 | 6.10 | 6.03 | 21 | 129.7 | 124.2 | 125.0 |
| 21 | 6.39 | 6.59 | 6.50 | 22 | 130.5 | 137.8 | 138.2 |
| 23 | 7.63 | N/A | N/A | 23 | 132.4 | 133.3 | 138.5 |
| 24 | 6.86 | 7.61 | 7.36 | 24 | 113.6 | 125.9 | 129.0 |
| 25 | N/A | 7.52 | 7.20 | 25 | 159.4 | 131.9 | 126.8 |
| 26 | 6.86 | 7.39 | 7.18 | 26 | 113.6 | 130.5 | 127.8 |
| 27 | 7.63 | 7.64 | 7.21 | 27 | 132.4 | 132.3 | 126.8 |
| 28[a] | 3.80 | N/A | 4.60 | 28[a] | 55.3 | 127.6 | 62.5 |

[a] Additional alkyl: 1, $OCH_3$; 2, $CF_3$; 3, $CH_2OH$.

were initially determined by rotation around dihedrals C13–C17–C20–C21 and C20–C21–C22–C23 (Figures 5–7).[14] The OH and $OCH_3$ groups were then rotated so as to find the lowest energy position. For **3**, hydrogen bonding between the 17-OH and 23-$CH_2OH$ group resulted in three pairs (**3a/3c**, **3b/3d**, **3e/3f**) of proton donor/acceptor conformers. The key dihedral angles for the lowest energy conformers, **1a–e**, **2a–f**, and **3a–h**, with energies within 6 kcal of the lowest energy conformer for **1–3**, are listed in Table 2. Conformers **1d**, **2d**, **3e**, and **3f**, which have an orthogonal alignment between the estradiol skeleton and the 17α substituent and an anti alignment between the phenyl ring and the C18 methyl, are referred to herein as anti orthogonal conformers. Conversely, conformers **1a**, **2a**, **3a**, and **3c** will be referred to as syn orthogonal conformers. Conformers **1b**, **2b**, **2c**, **3b**, **3d**, and **3h** are designated as extended conformers. All other conformers will be described via a combination of these names.

As the MM3 calculations show, significant changes in the 17α side chain conformation result in minor energy differences. In fact, most of the low-energy conformers are within 3 kcal of the lowest energy conformer. This made any conformational determination based purely on energy predictions unreliable.

More reliable conclusions regarding the 17α side chain conformation of **1–3** could be achieved by comparing predicted $^{13}$C chemical shifts for each MM3 conformer to experimental shifts. These predicted $^{13}$C chemical shifts, $\delta_{pred}$, were calculated by empirically scaling GIAO-calculated absolute shieldings, $\sigma$.[15] The appropriate scaling equation depends on the basis set. In this study, in which GIAO shielding calculations were obtained at the B3LYP/3-21G level with heteroatoms augmented at the 6-31+G* level, the appropriate scaling is given by eq 1,

$$\delta_{pred} = -1.168\sigma + 230.2 \quad (1)$$

(14) (a) Allinger, N. L.; Yuh, Y. H.; Lii, J. H. *J. Am. Chem. Soc.* **1989**, *111*, 8551, 8566, 8576. (b) *MM3(94)*; Tripos, Inc.: St. Louis, MO.

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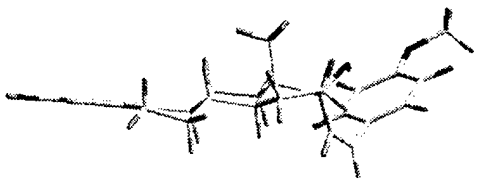
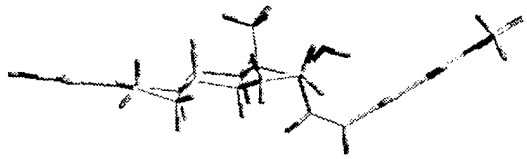
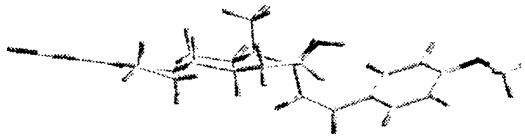


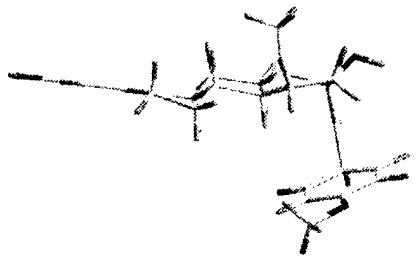
1a



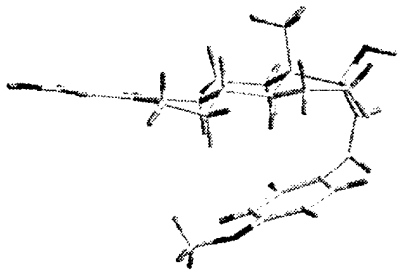
1b



1c



1d



1e

**Figure 5.** MM3-predicted geometries for the most stable conformers of **1**.

as determined previously.[16]All calculations were carried out with the Gaussian 98 program.[17] Tables 3–5 list the predicted $^{13}C$ chemical shifts of each MM3 conformer and the assigned experimental $^{13}C$ chemical shifts for **1**–**3**.

Previously, Dionne and Poirier showed that the carbons in the A, B, and C ring experience little shielding or deshielding effects from various 17α substituents since these carbons exhibit minor (~1 ppm) chemical shift changes. However, carbons in the D ring were significantly influenced by various 17α substituents. Specifically, C16 and C17 were shown to be the most heavily influenced. Our predicted $^{13}C$ chemical shifts correspond quite well with the carbons in rings A, B, and C (C1–C14); in fact, most of the $^{13}C$ predictions in rings A, B, and C are within 1 ppm of the assigned experimental values. These results demonstrate the accuracy of these predictions in an area of a well-defined geometry without any conformational distinction. The shielding and deshielding effects of the 17α substituent are clearly evident in the predicted chemical shift of C16 in different conformers of **1**. In conformers **1b** and **1e**, respectively the second lowest and the highest energy conformers of **1**, the predicted shifts of C16 differ by more than 8 ppm from the experimental value. Similarly for **2** and **3**, the predicted $^{13}C$ chemical shifts of C16 differ from the observed shift by more than 4 ppm for conformer **2d** and 5 ppm for conformers **2b**, **3f**, and **3h**. These large differences of the predicted shifts of C16 among similar conformers are attributed to the steric interactions between the ortho protons H23/27 and 16α. For example, the predicted C16 shift for extended conformer **1b** with a spatial distance between H23/27 and 16α of 2.2 D differs from experiment by more than 8 ppm, while the C16 shift prediction for anti orthogonal/extended conformer **1c** with a distance between H23/27 and 16α of 3.2 D is within 1 ppm of the experimental value.

(16) Forsyth, D. A.; Sebag, A. B. *J. Am. Chem. Soc.* **1997**, *119*, 9483.

(17) *Gaussian 98*, Revision A.3; Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian, Inc., Pittsburgh, PA, 1998.

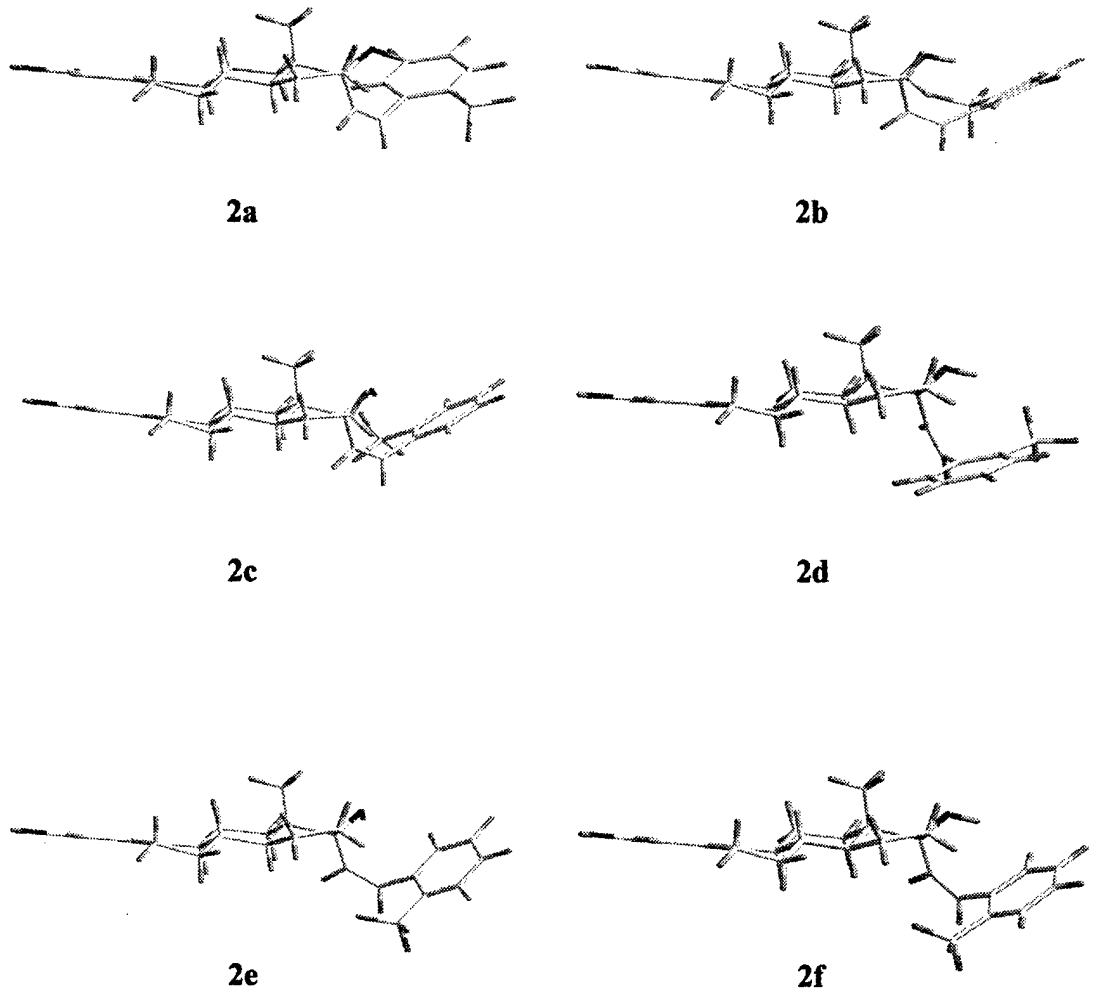



**Figure 6.** MM3-predicted geometries for the most stable conformers of **2**.

If **1**−**3** are rapidly exchanging among conformers, only average positions of the $^{13}C$ resonances will be observed experimentally on the NMR time scale. To determine the contributing conformers of **1**−**3,** we chose a statistical approach in which the predicted $^{13}C$ shifts of the C and D rings of all reasonable conformers of **1**−**3** were in each separate case treated as independent variables in a multiple independent variable regression analysis of the corresponding experimental data.[18] The predicted $^{13}C$ shifts of the A and B rings of all reasonable conformers of **1**−**3** were not used in this statistical analysis since they are all within 1 ppm of the experimental values regardless of the conformer. The regression analysis yielded fractional populations as the fitting parameters. All standard errors and confidence levels of the regression analysis were estimated using the Bootstrapping method.[19] The results and corresponding estimates of uncertainties (standard errors) are listed in Table 6. Both **1** and **2** were found to have a major conformer, **1c** 68(24)% and **2c** 60(1)%. Two minor conformers are also indicated for each: **1a** 20(12)% and **1d** 12(30)%, and **2a** 20(13)% and **2f** 20(8)%. For **3**, conformers **3a** 36(14)%, **3d** 34(26)%, and **3e** 28(14)% were found to be similarly populated. It is important to note that the large corresponding standard error of certain contributing conformers renders conclusions on their presence unreliable. This is evident with predicted conformer **1d** that is estimated to be 12% present but has a 30% standard error.

**NOESY Studies.** The solution state conformations of the 17α side chain of **1**−**3** were also probed by 2D and 1D NOESY experiments. In the case of **1**, the low-frequency region of the 2D NOESY spectrum reveals strong cross-peaks involving the vinyl proton, H20, with H14 and 12α,β. A weaker cross-peak with 16α could also be detected. The 2D NOESY spectrum also reveals weak cross-peaks between the H23/27 aryl protons and four alkyl protons, 12α, 12β, 16α, and 16β. The NOE data provide evidence for more than one conformer since no single conformer of **1** is expected to have an NOE with either H23 or H27 and both 12α and 16α. As all of the predicted low-energy conformers of **1** show, structures with a distance between H23 or H27 and 12α appropriate for an NOE preclude an NOE with 16α as a result of too great of a distance (>5 Å). Conformer **1c**, for example, which has a distance between H27 and 16α of 3.2 Å, has a distance greater than 5 Å between H23 or H27 and 12α.

In keeping with the 2D NOESY results for **1**, the selective 1D NOESY of H20 reveals equally strong enhancements of 12α,β and H14 and a weak enhancement of 16α (Figure 2c). Similarly, the 1D NOESY of H23/27 shows weak enhancement of 12α, 12β, 16α, and 16β (Figure 2d). Comparison of the intensity of these enhancements suggests a similarly short distance be-

(18) *SPSS*, V. 10, SPSS Inc.: Chicago, IL.
(19) Mooney C. Z.; Duval R. D. *Bootstrapping*; Sage Publications: Newbury Park, CA, 1993.

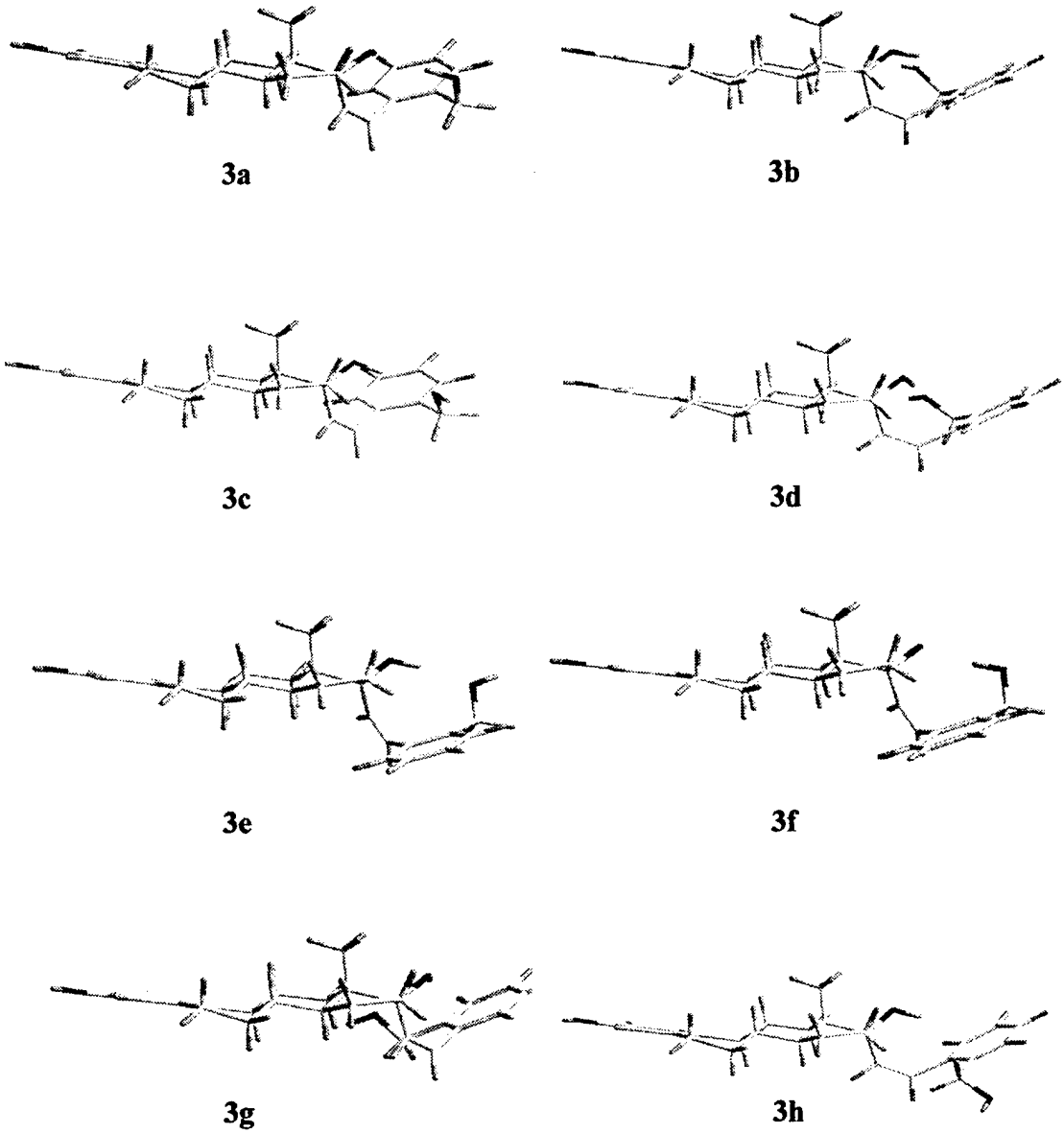



**Figure 7.** MM3-predicted geometries for the most stable conformers of **3**.

**Table 2. Relative Energies and Key Dihedrals of Predicted Conformers of 1–3 Using MM3**

| conformers | C13–C17–C20–C21 | C20–21–22–23 | rel energies (kcal/mol) |
|---|---|---|---|
| **1a** | −103 | −86 | 0 |
| **1b** | −156 | −68 | 0.6 |
| **1c** | −110 | −110 | 3 |
| **1d** | 105 | 86 | 3.2 |
| **1e** | 70 | 81 | 5.7 |
| **2a** | −112 | −99 | 0 |
| **2b** | −151 | 109 | 0.3 |
| **2c** | −148 | 93 | 1.7 |
| **2d** | 118 | 85 | 2.1 |
| **2e** | 162 | −125 | 2.3 |
| **2f** | 145 | −118 | 3.1 |
| **3a** | 106 | 90 | 0 |
| **3b** | 155 | 98 | 0.6 |
| **3c** | 109 | 94 | 2.3 |
| **3d** | 150 | 78 | 2.6 |
| **3e** | −131 | −81 | 3.3 |
| **3f** | −132 | −81 | 3.7 |
| **3g** | 111 | 105 | 4.2 |
| **3h** | 153 | 89 | 4.9 |

tween H20 and 12α,β and between H20 and H14, as well as greater distances between H20 and 16α and between H23/27 and 12α, 12β, 16α, and 16β. Table 7 summarizes and compares the intensity of the observed NOE signals with expected NOEs based on H–H distances in all predicted low-energy conformers of **1**. Comparison of these observed enhancements with expected NOE intensities for all predicted low-energy conformers of **1** rules out conformers **1d** and **1e** as contributing conformers based on the absence of observable NOE signals involving H23/27 with H14 and 15α. The strong, equally enhanced NOE signals between H20 and 12α and between H20 and H14 suggest that the major conformer bears an extended side chain geometry, consistent with conformers **1b** and **1c**. In comparing conformers **1b** and **1c**, the weak NOE signal between H23/27 and 16α is consistent with the expected weak NOE intensity between H23/27 and 16α of conformer **1c** and inconsistent with the expected strong NOE intensity between H27 and 16α of conformer **1b**. Therefore, conformer **1c** is considered the major conformer.

The weak NOE signal between H23/27 and 12α,β, which is not expected to arise from conformers **1b** or **1c** since these conformers have distances greater than 5 Å

**Table 3. Experimental and Predicted $^{13}C$ Chemical Shifts (ppm) of Predicted Conformers of 1 Using B3LYP/3-21G(X,6-31+G*)//MM3 Calculations**

| | 1a | 1b | 1c | 1d | 1e | expt |
|---|---|---|---|---|---|---|
| C1 | 127.5 | 127.6 | 127.5 | 127.5 | 127.4 | 126.9 |
| C2 | 113.0 | 113.0 | 113.1 | 113.0 | 112.8 | 113.5 |
| C3 | 152.9 | 152.9 | 153.0 | 152.9 | 152.6 | 155.8 |
| C4 | 115.6 | 115.6 | 115.7 | 115.6 | 115.7 | 115.8 |
| C5 | 136.3 | 136.2 | 136.1 | 136.1 | 136.1 | 138.3 |
| C6 | 31.0 | 31.1 | 31.1 | 31.0 | 30.7 | 29.9 |
| C7 | 28.4 | 28.4 | 28.4 | 28.5 | 27.0 | 28.5 |
| C8 | 40.1 | 39.8 | 39.7 | 39.6 | 38.7 | 40.7 |
| C9 | 44.3 | 44.4 | 44.3 | 44.3 | 42.5 | 44.5 |
| C10 | 132.1 | 132.1 | 132.2 | 132.3 | 132.3 | 131.9 |
| C11 | 28.5 | 28.4 | 28.5 | 28.5 | 28.5 | 27.3 |
| C12 | 34.2 | 32.0 | 31.9 | 32.8 | 34.0 | 32.6 |
| C13 | 48.6 | 48.0 | 47.6 | 48.4 | 49.8 | 48.7 |
| C14 | 50.7 | 48.7 | 49.1 | 49.0 | 47.3 | 49.9 |
| C15 | 26.0 | 26.8 | 26.1 | 25.3 | 27.1 | 23.7 |
| C16 | 39.8 | 46.6 | 38.1 | 37.5 | 46.6 | 38.3 |
| C17 | 86.1 | 83.4 | 79.7 | 83.0 | 86.1 | 83.8 |
| C18 | 16.0 | 15.3 | 14.3 | 15.1 | 16.1 | 14.5 |
| C20 | 142.2 | 144.5 | 142.4 | 142.9 | 152.0 | 135.1 |
| C21 | 133.1 | 130.8 | 134.8 | 135.3 | 134.5 | 129.7 |
| C22 | 127.8 | 129.6 | 130.4 | 129.5 | 132.1 | 130.5 |
| C23 | 129.2 | 130.1 | 127.4 | 131.7 | 129.1 | 132.4 |
| C24 | 117.1 | 118.8 | 119.2 | 117.1 | 118.7 | 113.6 |
| C25 | 157.5 | 157.5 | 157.6 | 156.9 | 157.4 | 159.4 |
| C26 | 109.4 | 110.0 | 110.8 | 109.2 | 110.1 | 113.6 |
| C27 | 132.2 | 128.6 | 128.8 | 129.6 | 130.9 | 132.4 |
| C28 | 54.0 | 54.0 | 54.6 | 54.0 | 54.5 | 55.3 |

**Table 4. Experimental and Predicted $^{13}C$ Chemical Shifts (ppm) of Predicted Conformers of 2 Using B3LYP/3-21G(X,6-31+G*)//MM3 Calculations**

| | 2a | 2b | 2c | 2d | 2e | 2f | expt |
|---|---|---|---|---|---|---|---|
| C1 | 127.3 | 127.6 | 127.6 | 127.5 | 127.6 | 127.4 | 127.4 |
| C2 | 113.0 | 113.1 | 113.1 | 113.0 | 113.0 | 113.0 | 113.9 |
| C3 | 153.0 | 153.0 | 152.9 | 153.0 | 152.9 | 153.2 | 155.0 |
| C4 | 115.9 | 115.8 | 115.6 | 115.7 | 115.8 | 115.7 | 116.2 |
| C5 | 136.0 | 136.0 | 135.9 | 136.1 | 136.3 | 136.3 | 139.1 |
| C6 | 30.9 | 31.0 | 30.9 | 31.1 | 31.1 | 31.1 | 30.7 |
| C7 | 28.3 | 28.4 | 28.4 | 28.4 | 28.1 | 28.0 | 28.7 |
| C8 | 39.7 | 39.7 | 40.0 | 39.8 | 39.9 | 40.1 | 41.2 |
| C9 | 44.1 | 44.0 | 44.4 | 44.1 | 43.9 | 44.0 | 45.0 |
| C10 | 131.9 | 132.1 | 132.1 | 132.0 | 131.9 | 132.0 | 131.9 |
| C11 | 28.5 | 28.4 | 28.3 | 28.6 | 28.4 | 28.6 | 27.7 |
| C12 | 34.9 | 32.1 | 33.9 | 32.3 | 30.8 | 30.9 | 33.7 |
| C13 | 48.0 | 47.9 | 49.3 | 48.0 | 47.7 | 48.0 | 49.0 |
| C14 | 50.1 | 49.5 | 50.5 | 49.1 | 49.1 | 48.7 | 50.8 |
| C15 | 26.5 | 26.8 | 26.3 | 26.3 | 25.8 | 26.1 | 24.4 |
| C16 | 42.6 | 45.1 | 39.3 | 35.0 | 40.6 | 36.1 | 39.3 |
| C17 | 86.1 | 84.4 | 87.8 | 81.8 | 81.8 | 80.5 | 85.8 |
| C18 | 14.6 | 15.1 | 16.1 | 14.7 | 15.4 | 15.0 | 14.8 |
| C20 | 143.7 | 147.0 | 141.6 | 142.0 | 145.5 | 140.6 | 138.7 |
| C21 | 127.8 | 126.2 | 129.2 | 129.4 | 132.0 | 133.3 | 124.2 |
| C22 | 138.9 | 138.9 | 135.5 | 139.8 | 139.2 | 140.5 | 137.8 |
| C23 | 129.2 | 133.4 | 131.5 | 132.4 | 131.1 | 130.5 | 133.3 |
| C24 | 125.9 | 127.2 | 127.4 | 126.6 | 129.2 | 128.1 | 125.9 |
| C25 | 130.7 | 128.8 | 131.1 | 130.6 | 130.8 | 130.4 | 131.9 |
| C26 | 131.7 | 131.0 | 130.2 | 131.4 | 132.0 | 131.7 | 130.5 |
| C27 | 132.2 | 128.8 | 134.5 | 129.4 | 129.6 | 130.8 | 132.3 |
| C28 | 127.0 | 127.1 | 127.4 | 126.7 | 127.3 | 127.0 | 127.6 |

between H23/27 and 12α,β, supports the presence of the syn orthogonal conformer **1a**.

In regard to conformations for **2**, the low-frequency region of the 2D NOESY spectrum of **2** displays a strong cross-peak between H20 and an overlapping region consisting of 12α, 12β, H14, and 15α. Additionally, weak cross-peaks between H27 and 12α,β, 16α, and 16β are observable. This pattern of NOESY cross-peaks is similar to that observed for **1**. An additional weak cross-peak between H21 and 12α,β could also be detected. A selective 1D NOESY of H20 reveals that the strong cross-peak

**Table 5. Experimental and Predicted $^{13}C$ Chemical Shifts (ppm) of Predicted Conformers of 3 Using B3LYP/3-21G(X,6-31+G*)//MM3 Calculations**

| | 3a | 3b | 3c | 3d | 3e | 3f | 3g | 3h | expt |
|---|---|---|---|---|---|---|---|---|---|
| C1 | 127.4 | 127.6 | 127.4 | 127.4 | 127.5 | 127.3 | 127.3 | 127.3 | 126.5 |
| C2 | 113.1 | 113.2 | 112.9 | 112.9 | 112.8 | 113.0 | 112.9 | 113.0 | 113.2 |
| C3 | 153.1 | 153.0 | 152.8 | 152.9 | 152.8 | 153.1 | 152.9 | 152.9 | 153.2 |
| C4 | 115.9 | 115.7 | 115.7 | 115.7 | 115.6 | 115.8 | 115.7 | 115.8 | 115.2 |
| C5 | 136.0 | 136.0 | 136.1 | 136.3 | 136.3 | 136.3 | 136.1 | 136.1 | 137.5 |
| C6 | 30.9 | 31.0 | 31.0 | 31.1 | 31.2 | 31.0 | 31.1 | 31.0 | 29.8 |
| C7 | 28.2 | 28.5 | 28.4 | 28.5 | 28.3 | 28.1 | 28.5 | 28.4 | 27.9 |
| C8 | 39.8 | 39.7 | 39.6 | 40.0 | 40.0 | 40.1 | 40.1 | 39.6 | 40.2 |
| C9 | 44.0 | 44.1 | 44.2 | 44.4 | 44.0 | 43.8 | 44.5 | 44.3 | 44.0 |
| C10 | 131.7 | 131.8 | 132.5 | 132.3 | 132.4 | 131.4 | 132.2 | 132.1 | 131.9 |
| C11 | 28.4 | 28.3 | 28.6 | 28.5 | 28.7 | 28.4 | 28.6 | 28.5 | 26.8 |
| C12 | 34.3 | 31.3 | 34.9 | 31.9 | 31.7 | 30.4 | 33.0 | 32.3 | 33.0 |
| C13 | 48.0 | 47.5 | 47.8 | 48.0 | 47.7 | 48.1 | 49.1 | 48.3 | 48.0 |
| C14 | 50.4 | 49.2 | 50.1 | 49.2 | 49.3 | 49.1 | 50.8 | 49.3 | 49.9 |
| C15 | 26.2 | 26.6 | 26.8 | 26.1 | 25.7 | 25.0 | 26.2 | 26.7 | 23.4 |
| C16 | 39.4 | 44.9 | 43.3 | 42.0 | 34.4 | 30.9 | 39.2 | 43.6 | 38.4 |
| C17 | 85.9 | 83.3 | 85.4 | 84.4 | 79.9 | 80.2 | 87.3 | 85.8 | 84.8 |
| C18 | 16.1 | 15.3 | 14.7 | 16.0 | 15.5 | 15.4 | 16.0 | 14.9 | 14.6 |
| C20 | 141.6 | 144.6 | 145.0 | 146.1 | 142.8 | 136.2 | 141.7 | 141.5 | 138.0 |
| C21 | 130.9 | 129.6 | 128.3 | 127.8 | 129.2 | 136.5 | 130.5 | 127.8 | 125.0 |
| C22 | 134.5 | 136.4 | 140.2 | 140.6 | 140.5 | 135.5 | 133.4 | 134.3 | 138.2 |
| C23 | 141.0 | 140.6 | 133.2 | 133.5 | 135.9 | 140.4 | 136.4 | 140.0 | 138.5 |
| C24 | 131.9 | 131.7 | 131.4 | 131.2 | 132.5 | 131.8 | 130.0 | 130.4 | 129.0 |
| C25 | 128.5 | 126.8 | 128.5 | 126.9 | 126.9 | 127.9 | 127.7 | 127.9 | 126.8 |
| C26 | 126.0 | 128.3 | 127.0 | 128.8 | 128.3 | 126.2 | 126.2 | 126.6 | 127.8 |
| C27 | 131.9 | 131.7 | 131.4 | 131.2 | 127.7 | 131.8 | 132.8 | 126.5 | 126.8 |
| C28 | 64.5 | 65.0 | 65.9 | 66.2 | 64.7 | 64.1 | 63.2 | 63.3 | 62.5 |

**Table 6. Summary of the Multiple Independent Variable Regression Analysis[a] of the Calculated $^{13}C$ Shifts of Predicted Conformers of 1–3**

| conformer | estimate (%) | standard error (%) |
|---|---|---|
| **1a** | 20 | 12 |
| **1b** | 0 | 7 |
| **1c** | 68 | 24 |
| **1d** | 12 | 30 |
| **1e** | 0 | 0 |
| **2a** | 20 | 13 |
| **2b** | 0 | 15 |
| **2c** | 60 | 1 |
| **2d** | 0 | 7 |
| **2e** | 0 | 11 |
| **2f** | 20 | 8 |
| **3a** | 36 | 14 |
| **3b** | 0 | 1 |
| **3c** | 0 | 5 |
| **3d** | 34 | 26 |
| **3e** | 28 | 14 |
| **3f** | 0 | 1 |
| **3g** | 2 | 7 |
| **3h** | 0 | 10 |

[a] Constraints: Each conformer is greater than or equal to 0%. Conformer sets **1a–e**, **2a–f**, and **3a–h** are equal to 100%.

consists mainly of signal from H14 with some contribution from 12α,β (Figure 3c). The 1D NOESY of H20 also displays a very weak enhancement of 16α. The 1D NOESY of H27 displays the expected weak enhancements of 12α,β, 16α, and 16β expected from the 2D NOESY experiment (Figure 3d). The NOE data indicates the presence of at least two conformers with rotated phenyl rings since no predicted conformer of **2** is expected to have an NOE with H27 and both 12α and 16β.

As described in detail below, comparing these observed enhancements with expected NOE intensities for predicted conformers of **2** suggests that conformer **2c** is the major conformer with minor contribution from **2a** and other conformers as well (see Table 8).

**Table 7. Summary and Comparison of Observed NOE Enhancements with Expected NOE Intensities[a] for Predicted Conformers of 1**

| irradiated | enhanced | 1a | 1b | 1c | 1d | 1e | expt |
|---|---|---|---|---|---|---|---|
| H20 | 12α,β | w | s | s | s | w | s |
| H20 | H14 | s | s | s | w | w | s |
| H20 | 16α | s | w | w | w | w | w |
| H23/27 | 12α,β | s | n | n | w | s | w |
| H23/27 | H14 | n | n | n | s | s | n |
| H23/27 | 15α | n | n | n | w | s | n |
| H23/27 | 16α | n | s | w | s | s | w |
| H23/27 | 16β | n | w | w | w | s | w |

[a] Expectations of strong (s), weak (w), and no (n) NOE enhancements correspond to H−H distances of 0−2.99, 3.0−4.99, and >5 Å.

**Table 8. Summary and Comparison of Observed NOE Enhancements with Expected NOE Intensities[a] for Predicted Conformers of 2**

| irradiated | enhanced | 2a | 2b | 2c | 2d | 2e | 2f | expt |
|---|---|---|---|---|---|---|---|---|
| H20 | 12α,β | w | s | s | s | s | s | s |
| H20 | H14 | s | s | s | w | s | w | s |
| H20 | 16α | s | w | w | w | w | w | w |
| H21 | 12α,β | w | n | n | n | n | n | w |
| H27 | 12α,β | s | n | n | w | n | n | w |
| H27 | H14 | n | n | n | s | n | n | n |
| H27 | 15α | n | n | n | s | n | n | n |
| H27 | 16α | n | s | w | w | n | s | w |
| H27 | 16β | n | w | w | n | w | w | w |

[a] Expectations of strong (s), weak (w), and no (n) NOE enhancements correspond to H−H distances of 0−2.99, 3.0−4.99, and >5 Å.

The observed strong and moderately strong enhancements of H14 and 12α,β, respectively, upon irradiation of H20 suggests that the 17α side chain of the major conformer bears an extended geometry with a closer distance between H20 and H14 than between H20 and 12α,β. This is only consistent with conformers **2b** and **2c**, which have distances between H20 and H14 of 2.0 and 2.2 Å and between H20 and 12α of 2.1 and 2.5 Å, respectively. Comparing **2b** and **2c**, the weak enhancement of 16α upon irradiation of H27 is consistent with the expected weak NOE intensity between H27 and 16α of conformer **2c** but is inconsistent with the expected strong NOE intensity between H27 and 16α of conformer **2b**. Conformer **2c** thus is considered the major conformer.

As for minor conformers, conformer **2d** can be ruled out as a contributing conformer because of the absence of an observable NOE between H27 and H14 or 15α. For conformer **2f**, the expected weak enhancement of H14 upon irradiation of H20 suggests only a minor contribution since the observed enhancement is strong. The geometrically similar conformer, conformer **2e**, could not be ruled out with NOE data as a minor conformer. The presence of the syn orthogonal conformer **2a** is clear from the NOE enhancement of 12α,β upon irradiation of H27. All other conformers of **2** have a distance between H27 and 12α,β greater than 5 Å. The NOESY cross-peak between H21 and 12α,β further supports the presence of conformer **2a** since all other predicted conformers bear a distance between H21 and 12α,β greater than 5 Å.

The low-frequency region of the 2D NOESY spectrum of **3** displays additional cross-peaks not found in the similarly patterned 2D NOESY of **1** and **2** (Figure 8). Aside from the cross-peaks between H20 with 12α,β, H14, and 16α and H27 with 12α,β and 16α analogous to those observed for **1** and **2**, additional weak cross-peaks between H21 and H27 with an overlapping region consisting of H14 and 15α appear. Also, weak cross-peaks between the methylene protons of the 23-$CH_2OH$ group and 12α,β and 16α are observable. A selective 1D NOESY of H20 reveals strong enhancements of H14 and 12α,β and weak enhancement of 16α (Figure 4c). The 1D NOESY of H27 displays the expected weak enhancements of 16α and the overlapped regions consisting of 12α,β and H14,15α (Figure 4d).

Comparing these observed enhancements with expected NOE intensities for predicted conformers of **3** indicates the presence of at least three conformers (see Table 9). The observed weak NOE enhancements of H21 with H14 and H27 with the overlapped region consisting of H14 and 15α are only consistent with the two predicted anti orthogonal conformers **3e** and **3f**. All other conformers of **3** have a distance between these protons greater than 5 Å. Similarly, the observed weak NOE enhancements of H21 with 12α,β and H27 with 12α,β are only consistent with the two syn orthogonal conformers **3a** and **3c**. The very weak enhancement between the 23-$CH_2OH$ methylene protons and 12α,β is only consistent with the predicted syn orthogonal/extended conformer **3g**.

As for the extended conformers, **3b** and **3d**, the strong NOE enhancements of 12α,β and H14 upon irradiation of H20 would be consistent with their presence. However, these strong NOE enhancements could reasonably result from an averaged contribution of the syn orthogonal conformers **3a** and **3c**, the anti orthogonal conformers **3e** and **3f**, and the syn orthogonal/extended conformer **3g**. Thus, other reasonable interpretations of the NOE data are feasible. The remaining extended conformer, **3h**, cannot be ruled out with NOE data, but the expected strong enhancement of 16α,β upon irradiation of the methylene protons of the 23-$CH_2OH$ group suggests only a minor contribution.

## Discussion

The NOE data indicate that **1**−**3** each exist in solution as an equilibrating mixture of conformers. Unlike **3**, both **1** and **2** show the dihedral C18−C17−C20−C21 restricted to a similar range of rotation. For **1** and **2**, the position of the 17α side chain ranged from the syn orthogonal conformers **1a** and **2a** to the anti orthogonal/extended conformers **1c** and **2e**, whereas for **3**, the 17α side chain ranged from the syn orthogonal conformers **3a/3c** to the anti orthogonal conformers **3e/3f**. In particular, the NOE data indicate that **1d** and **2d**, which are analogous to **3e/3f** in side chain position, are not populated. Although the 17α side chain of **1** and **2** appears to have a similar range of rotation, the NOE data do suggest that the relative populations of the major conformers of **1** and **2** are slightly different. For **1**, the NOE data indicates that the major conformer **1c** bears an anti orthogonal/extended 17α side chain, whereas for **2**, the major conformer **2c** has an extended 17α side chain. As for minor conformers, the NOE data suggests that the syn orthogonal conformer **2a** is more abundant in solution for **2** than **1a** is for **1.** This conclusion is rationalized from the H21, 12α,β cross-peak found only in the 2D NOESY of **2**.

The presence of the anti orthogonal conformers only found in **3** can be explained by stabilization experienced by **3e** and **3f** as a result of hydrogen bonding between the 17-OH and 23-$CH_2OH$ groups. For **3**, intramolecular hydrogen bonding is not predicted for any of the other conformers according to the MM3 calculations.

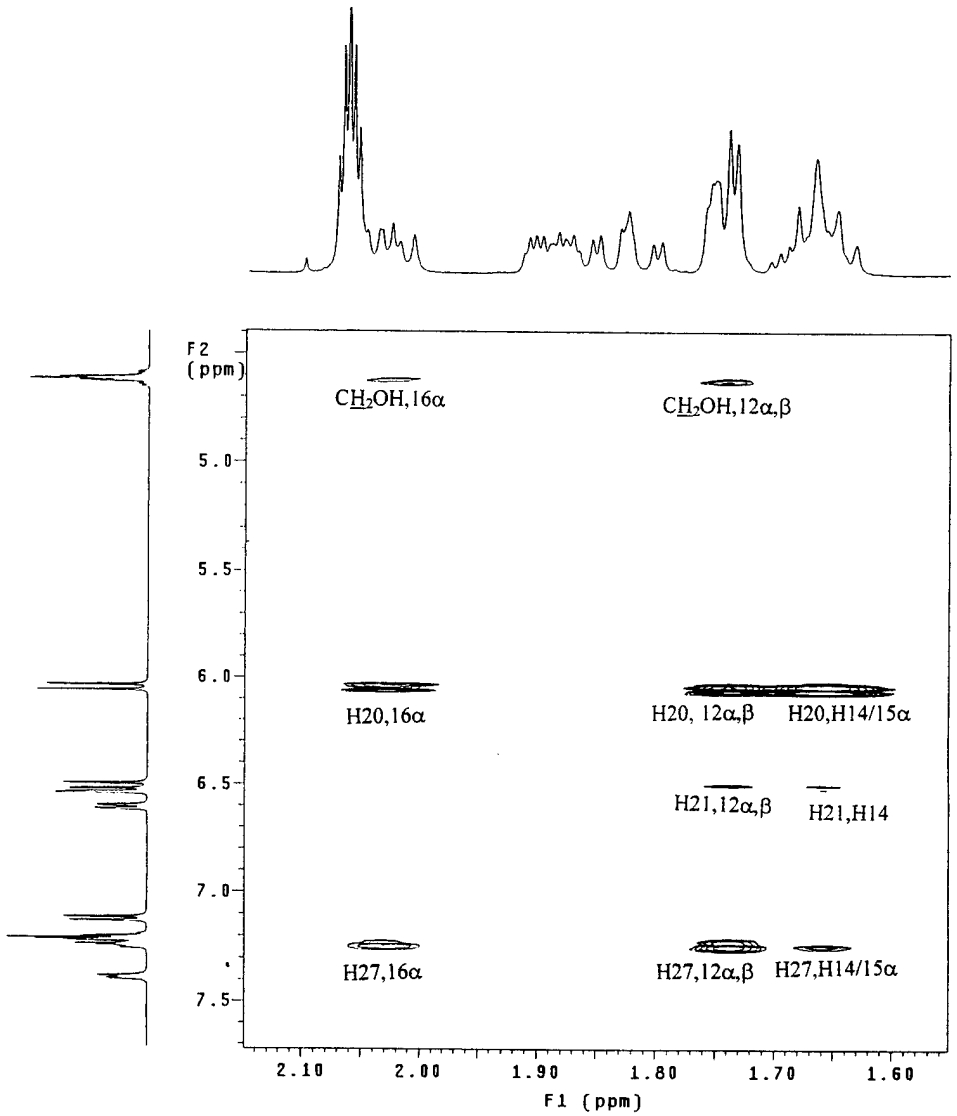



**Figure 8.** Spectral region of a 500 MHz 2D NOESY spectrum of **3** obtained with a mixing time of 500 ms. The NOE connectivities are indicated.

**Table 9. Summary and Comparison of Observed NOE Enhancements with Expected NOE Intensities[a] for Predicted Conformers of 3**

| irradiated | enhanced | 3a | 3b | 3c | 3d | 3e | 3f | 3g | 3h | expt |
|---|---|---|---|---|---|---|---|---|---|---|
| H20 | 12α,β | w | s | w | s | s | s | s | s | s |
| H20 | H14 | s | s | s | s | w | w | s | s | s |
| H20 | 16α | s | w | s | w | w | w | s | w | w |
| H21 | 12α,β | w | n | w | n | n | n | n | n | w |
| H21 | H14 | n | n | n | n | w | w | n | n | w |
| H27 | 12α,β | s | n | s | n | n | n | n | n | w |
| H27 | H14 | n | n | n | n | w | w | n | n | w |
| H27 | 15α | n | n | n | n | w | w | n | n | w |
| H27 | 16α | n | s | n | s | w | w | w | n | w |
| $CH_2OH$ | 12α,β | n | n | n | n | n | n | s | n | w |
| $CH_2OH$ | 16α | w | n | w | n | w | w | n | s | w |

[a] Expectations of strong (s), weak (w), and no (n) NOE enhancements correspond to H–H distances of 0–2.99 3.0–4.99, and >5 Å.

The NOE data are mostly consistent with our statistical approach of evaluating contributing conformers from predicted $^{13}C$ shifts. The findings from multiple independent variable linear regression analysis of the $^{13}C$ data of **1** and **2**, that the major conformers **1c** and **2c** are 68% and 60% populated and that the minor conformers **1a** and **2a** are both 20% populated, are compatible with the identities of major and minor conformers favored by NOE data. Additionally for **3**, a 36% populated syn orthogonal conformer **3a**, 34% populated extended conformer **3d**, 28% populated anti orthogonal conformer, and 2% populated syn/extended conformer **3g** is quite consistent with the NOE data.

Consistent with the NOE data, the statistical analysis suggests that conformers **1b**, **1e**, and **2d** are not found in solution. For **1**, although a 12% contribution of conformer **1d** is inconsistent with the NOE data, perhaps this is only a minor inconsistency since the identity of the major conformer and another minor conformer are consistent in the two methods. Furthermore, for **2**, a 20% population of conformer **2e** is consistent with the NOE data, although the NOE data do not clearly indicate that **2e** is the only additional minor conformer that is populated.

## Conclusions

This study reveals that the substituent on the phenyl group of the 17α,*Z*-phenylvinyl substituent of estradiols can affect the conformational equilibrium of the 17α side chain. Hydrogen bonding stabilization between the 17-

OH and a 23-$CH_2OH$ substituent of **3** results in an additional anti orthogonal conformer not found in **1** or **2**. The similarity in solution conformations of **1** and **2** suggests they occupy a similar receptor volume that is consistent with their similar RBA of 20 and 23 at the estrogen receptor. The different conformational equilibria of **3** may explain its significant RBA of 140, which is greater than estradiol itself. Other effects such as hydrogen bonding, size, and electronic effects of the substituents may also play roles. These results can be applied to the design of subsequent ligands which will examine these conformational and substituent effects.

## Experimental Section

HMQC, COSY, 1D and 2D NOESY spectra were obtained on a Varian Unity INOVA instrument at 500 MHz. DEPT and $^{13}C$ spectra were obtained on a Varian Mercury instrument at 300 MHz.

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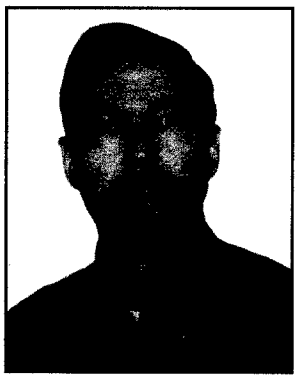
JO000806H

# Synthesis of Auger Electron-Emitting Radiopharmaceuticals



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**Abstract**: Targeted radiotherapy using Auger electron-emitting pharmaceuticals offers both advantages and challenges compared to alternative α - or β -emitting agents. The low energy Auger electrons deposit their energy within the target cell thereby minimizing collateral damage. To achieve this effect, however, the radiopharmaceutical must incorporate the appropriate radionuclide, be efficiently synthesized, and once administered, be distributed selectively to its biological target. This review covers the synthesis of agents which have prepared over the past decade either as Auger electron-emitting radiopharmaceuticals or which have the potential as such. While not an exhaustive review, the major classes of agents, such as hormone receptor ligands, nucleoside analogs and intercalating agents are described.

## I.INTRODUCTION

Targeted radiotherapy, using internally emitted radiation, offers an alternative to the use of traditional radiation therapy or boron neutron capture therapy. The key features in this modality include the ability to direct the agent to the target tissue using a biological marker, the deposition of high linear energy transfer (LET) radiation at the site in a short period of time, and to have that energy transfer result in a localized cytotoxic event. The result of this process is to cause a high lethality rate among targeted populations of cells, often neoplastic cells, while generally sparing neighboring normal or nontargeted cells. Aspects of this process, e.g., use of antibodies and oligonucleotides to target cells, microdosmetry and the use of alpha-emitting radionuclides, are discussed in accompanying reviews in this issue.

Unlike β- or α-emitting radionuclides, which deposit their LET effects over several cell diameters, the low energy Auger electrons emitted during radioactive decay deposit their energy within subcellular dimensions [1-3]. As a result, for a compound labeled with an Auger electron-emitting radionuclide to exert a cytotoxic effect, it has to be able to penetrate within the cell. In addition, for the agent to generate a lethal event, that localization should be within the proximity of the nuclear DNA. As described elsewhere, and previously reported, cell death is associated most closely with the ability to cause double strand breaks in the DNA as a consequence of the shower of low energy electrons. Therefore, for an Auger electron-emitting radiopharmaceutical to have therapeutic potential, 1. a radionuclide must have an appropriate radiation decay profile, 2. a radionuclide should be able to be economically prepared in reasonably high specific activity and purity, 3. a radionuclide should be incorporated efficiently into a carrier molecule, 4. a carrier molecule should display biodistributional selectivity for the target tissue, and 5. in the target tissue, the agent should associate with the nuclear DNA complex for a time consistent with the half-life of the radionuclide. To date, virtually no Auger electron-emitting radiopharmaceutical has met all of these criteria. However, sufficient data both from *in vitro* studies with putative Auger emitters and from α/β/γ-emitting radiopharmaceuticals suggest that success may be achieved with improved targeting mechanisms.

Based on the previously listed criteria, one is left with a relatively small set of available radionuclides with which to work (Table 1).

**Table 1. Auger Electron-Emitting Radionuclides for Use in Radiopharmaceutical Synthesis**

| | | |
|---|---|---|
| Chromium-51 | Gallium-67 | Bromine-77, 80m |
| Indium-111 | Iodine-123, 125 | Platinum-193m |
| Thallium-201 | | |

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The most prominent of the Auger emitting radionuclides are the isotopes of iodine (I-125 and I-123) and bromine (Br-77 and 80 m). To a much less degree, studies have been reported related to the Auger effects of In-111 and Pt-193m. The other radionuclides that emit Auger electrons as part of their decay scheme, however, either have other emissions (γ, β+, β-), half-life considerations or production characteristics that preclude their use as potential Auger-radiotherapeutics. The chemical properties of the radiohalogens allow them to be more readily incorporated into organic molecules by traditional synthetic methods, whereas the metal ions require chelation techniques [4,5]. These two strategies, as shown later, influence the types of targeting agents to which they are bound.

The low energy of the Auger electrons requires that they be emitted as close to the nucleus of the cell as possible to exert their lethal effect. Therefore, the carrier molecule for the radionuclide has to cross the cell membrane either by passive diffusion or via a specific carrier mediated process. Once inside the cell, the carrier-radionuclide complex has to bind selectively to the DNA or a DNA associated protein. This criterion dramatically reduces the number of potential carriers available for molecular manipulations (Table 2).

**Table 2. Mechanisms for Nuclear/Intracellular Localization**

| | |
|---|---|
| 1. | Nuclear Receptor Binding |
| 2. | DNA-directed Agents |
| 3. | Other Intracellular Targets |

For the treatment of cancers with Auger emission radiotherapy, the most promising carrier molecules are the steroid hormones (via their receptors), DNA directed agents (nucleosides, intercalators, groove binding) and a few proteins and peptides [6]. Given the available radionuclides, there are relatively few options to exploit. This is in a distinct contrast to those β- or α-emitting agents which do not require that degree of localization.

The primary objective of this review is to cover the progress since 1990 [7] in the preparation of radiotherapeutic agents bearing (potential) Auger electron-emitting radionuclides. Because the biophysical constraints imposed on this approach have limited its utility, a secondary objective will be to consider potential agents, based on work done with other radiodiagnostic or radiotherapeutic materials.

## II. HORMONE RECEPTOR LIGANDS

The mechanism of action of the steroid hormones has made the preparation of labeled analogs one of the major foci of radiopharmaceutical development. Receptors for the endogenous hormones are overexpressed in a number of human carcinoma cell lines. The circulatory steroids enter all cells by passive diffusion, however, only responsive cells contain the requisite hormone receptor. Binding of the hormone to its cognate receptor in the nucleus of the cell initiates a series of events which includes the binding of the steroid-receptor complexes to the nuclear DNA. The high affinity for the receptor, the selectivity of the hormone-receptor interactions, and the avidity of the complex for the DNA combine to provide the basis for radiotherapy using Auger electron-emitting steroid hormone receptor ligands [8]. Although success in achieving the affinity and selectivity for the estrogen receptor has been the greatest, synthesis of radiolabeled androgen and progestin receptor ligands have been reported in the past 10 years.

### A. Estrogen Receptor Ligands

During the 1980's the synthesis of a number of radiohalogenated analogs of estradiol were reported. The reviews by Katzenellenbogen [9] and Cummins [10] describe the labeling methods and biological properties of many of these ligands. While most of the emphasis was focused on the radiodiagnostic potential of these agents, the presence of Auger electrons from the decay of I-123/125 and Br-77/80 m initiated interest in their radiotherapeutic applications. The compounds that were most extensively evaluated were the 16α-halogenated (I/Br)-estradiols and the 17α-halo (I/Br) vinyl estradiols. The former were prepared by nucleophilic displacement of the appropriately substituted 16β- X-estradiol. The latter were synthesized using the radiohalodestannylation methodology that we developed in the early 1980's. Both methods provided target compounds rapidly and in high yields (Fig. **1**). Studies with these agents demonstrated that the presence of the halogen at either position was tolerated or, in the case of the 17α-halovinyl estrogens, beneficial to binding. Additional substituents at the 11β or 7α positions also enhanced receptor binding. *In vitro* studies indicated that radiocytotoxicity was receptor mediated and, therefore, validated this approach.

More recent synthetic approaches have focused on two aspects, the enhancement of affinity within the estradiol structure, or identification of

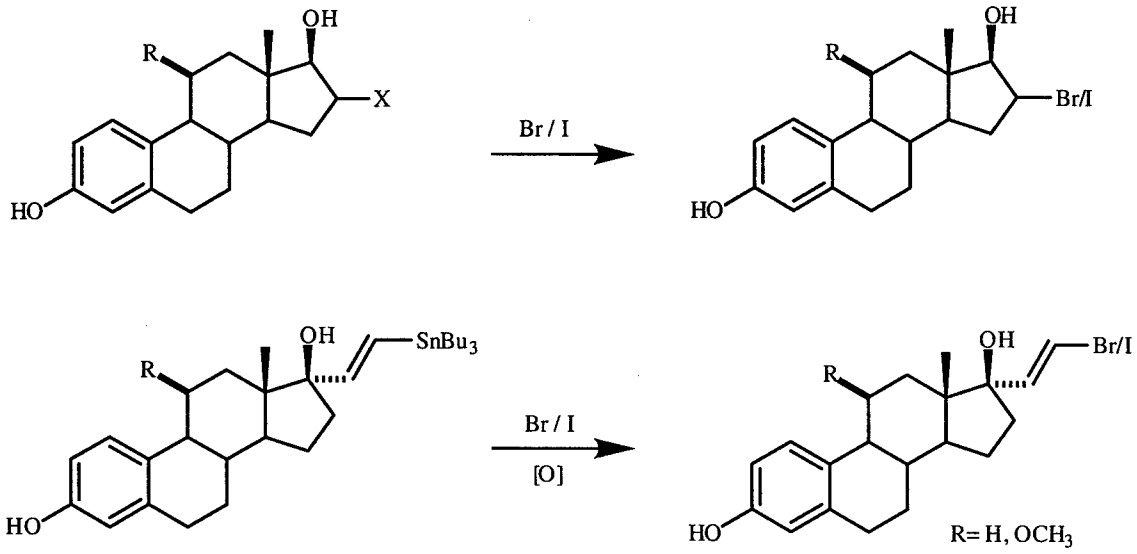


**Fig. (1).** Radiobromination/iodination of estradiols.

nonsteroidal estrogens with possibly better pharmacokinetic properties. Because both approaches utilized the destannylation methodology for introduction of the Auger emitting radiohalides, the challenges were primarily associated with the synthesis of the precursor trialkylvinylstannanes. Previous studies [11] had demonstrated that the 17α-Z-halovinyl estradiols had higher affinity than the corresponding 17α-E-isomers. Small lipophilic substituents at the 11β-position provided an additional enhancement of relative binding affinity (RBA) [12]. The synthesis of the 11β-vinyl/ethyl 17α-Z-tributylstannylvinyl estradiol precursor for radiohalogen labeling is shown in Fig. **2**. The process involved at least 13 steps with an overall yield of <2%, prior to the radiohalogenation (the E-isomer can be obtained in ~4% overall yield). As a result, few of these analogs have been evaluated *in vitro* or *in vivo*. Initial data suggest that the radiocytotoxicity is retained, however, the physicochemical properties of the individual compounds produce variations in the pharmacokinetics. Additional work by Cummins [13] and Quincy [14] have also utilized the 17α -iodovinyl group to prepare labeled estrogenic ligands, although with imaging as the objective.

The alternate approach for estrogen receptor ligands utilizes a nonsteroidal structure. DeSombre, *et al.* prepared the [Br-80m] labeled bis(hydroxyphenyl)ethylene [15]. While initially prepared via direct radiobromination of the

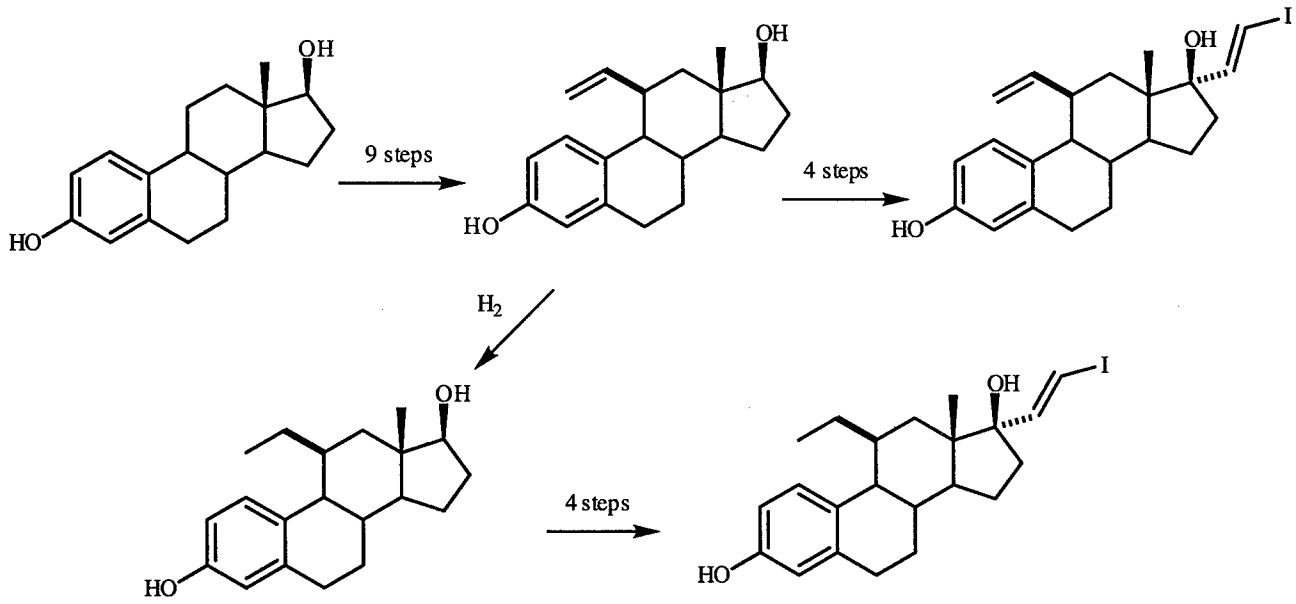


**Fig. (2).** Synthesis of 11β-substituted estradiols.

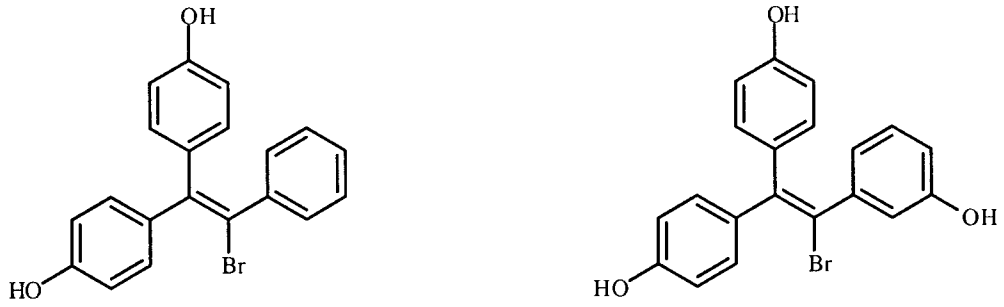


**Fig. (3).** Bis- and tris-hydroxy-triphenylethylene bromide.

protected material, better yields of purer product were obtained by using the destannylation methodology. Comparison with the 11β-substituted 17α-iodovinyl estradiols suggested that some pharmacokinetic advantages were associated with the nonsteroidal structure. In order to improve receptor binding, an analog with an additional phenolic group has been prepared (Fig. **3**). The initial synthesis of the stannyl intermediate was achieved using transmetallation of the vinyl bromide with alkyl lithium followed by quenching with trialkyltin halide, however, the yield in the final step was low. Use of hexabutylditin and Pd(0)catalyst raised the yield by an order magnitude. Biological studies with these labeled products (Br-80m/I-123) are currently undergoing *in vitro* evaluation.

An alternate approach to the use of labeled estrogenic agonists is the preparation of antagonists. Although both steroidal and nonsteroidal antagonists have been described in the literature, only labeled derivatives of nonsteroidal antagonists have been reported. For example, iodoxifene has been prepared and evaluated as a selective estrogen receptor modulator (SERM) and

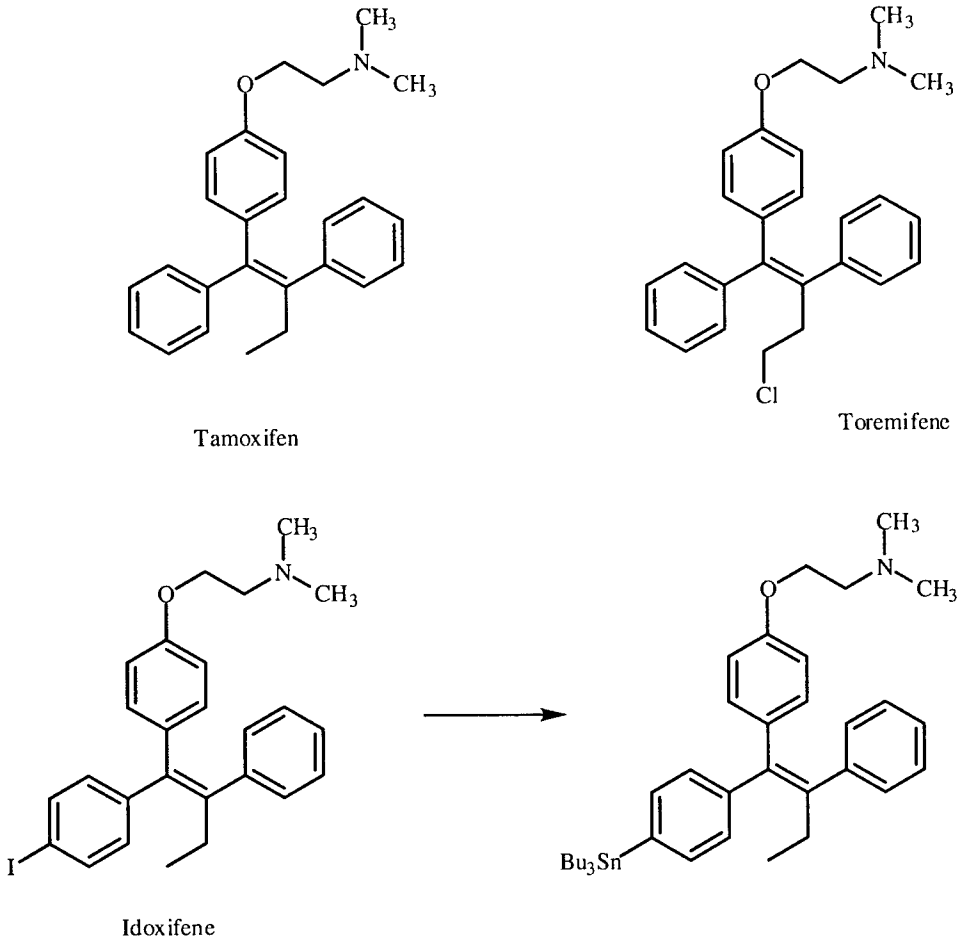


**Fig. (4).** Nonsteroidal estrogen receptor ligands (antiestrogens).

its resynthesis with the addition step for replacement of iodine by tributyltin would provide the immediate precursor for labeling with either of the isotopes of iodine (Fig. **4**).

## B. Progesterone Receptor Ligands

The design of radiolabeled progesterone receptor seeking ligands, as described by Brandes and Katzenellenbogen, has been hampered by several factors [16,17]. A major problem is that the endogenous ligand, progesterone, has a binding affinity for its receptor that is almost an order of magnitude less than that of estradiol for the estrogen receptor, 4.5 x $10^{-9}$M vs. 3 x $10^{-10}$M. As a consequence, a ligand receptor complex is less likely to remain associated with the nuclear DNA long enough for therapeutically relevant Auger emitting radionuclides to deposit their energy at the site. In addition, structure-activity studies on the progesterone receptor ligands provided relatively few examples of compounds that had relative binding affinities (RBA) significantly greater than progesterone itself. Among that subset, even fewer were amenable to radiolabeling at sites that would be chemically or metabolically stable (Fig. **5**). During the 1980's Hochberg, *et al.* described the preparation of the 17α-iodovinyl testosterone (ethisterone) and 19-nortestosterone (norethisterone) analogs in their radiolabeled form using the halodestannylation methodology [18,19]. The Schering group also explored these as potential ligands for the progesterone receptor [20].Salman, *et al.* introduced the radiohalogen at the terminus of a 17α-haloalk-1-ynyl-19-nortestosterone in an attempt to enhance the affinity of the compound for the receptor [21]. While these compounds were chemically stable and relatively resistant to metabolism, they displayed little ability to localize in progesterone receptor rich tissue, to be retained there or exert any radiocytotoxic effect.

Since 1990, most of the efforts in the area have focused on the radiodiagnostic applications of the labeled progestins [22]. A number of the syntheses, however, employed labels that could be considered for radiotherapy given the appropriate radionuclide. Examples of these syntheses are shown in Fig. **6** and the putative radiosynthesis with the Auger emitting nuclide is provided. Van Lier's group synthesized the 17α -iodovinyl testosterone and 19-nortetstosterone derivatives and evaluated their radioiodinated forms as ligands for the progesterone (and androgen) receptors [23]. Their results essentially confirmed previous findings regarding the inadequacy of the ligands.

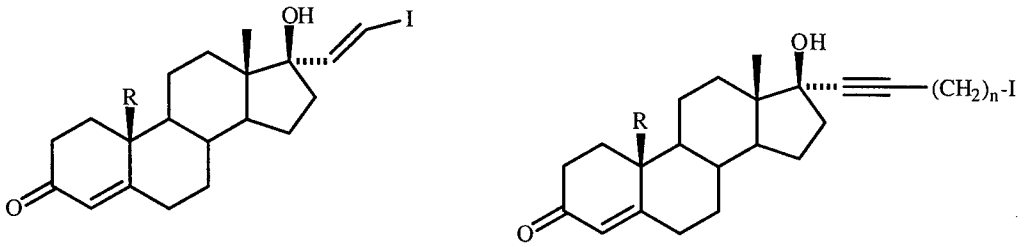


**Fig. (5).** Radiolabeled Derivatives of ethisterone and norethisterone.

Based on the studies of Brandes and Katzenellenbogen which were primarily directed to F-18-labeled progesterone ligands, Van der Bos and Rijks prepared and evaluated a series of four iodinated progestins [24,25]. Two were the E- and Z-isomers of 17α-iodo-19-nortestosterone previously evaluated, two were the E- and Z-isomers 17β-hydroxy-17α-iodovinyl-11-

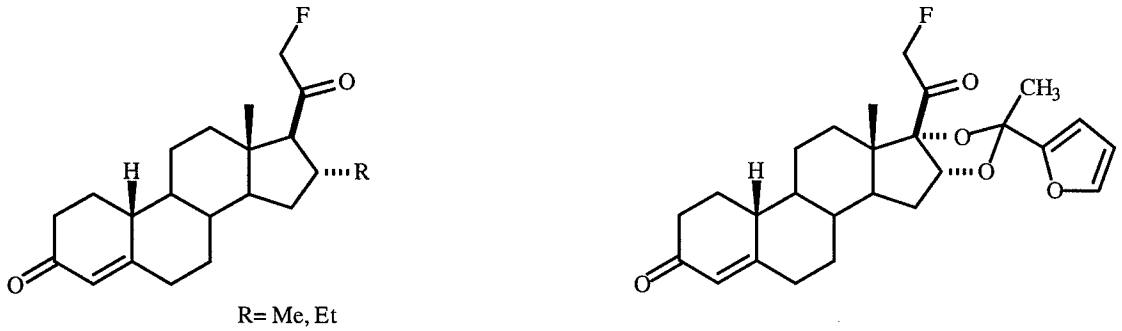


**Fig. (6).** Radiofluorinated progesterone receptor ligands.

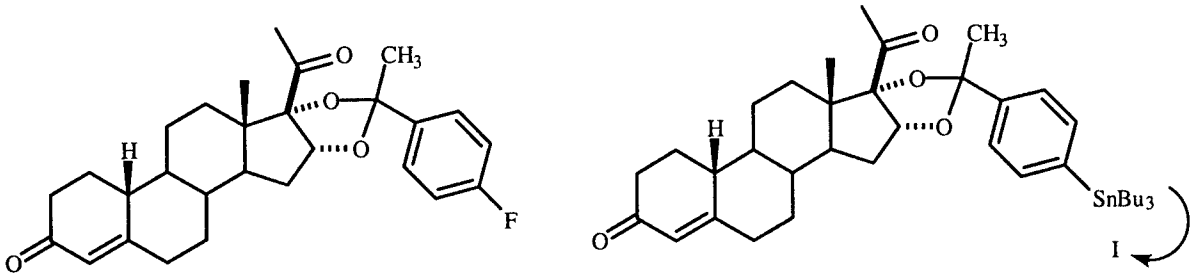


**Fig. (7).** Use of tributylstannyl analog as precursor for iodine radionuclide.

methylene-19-norgon-4-ene-3-one [ORG 3236 analogs), and the 21-iodophenoxy-16-α-ethyl-19-norpreg-4-ene-3,20-dione (ORG-2058 analog). The two ORG 3236 compounds had RBA values significantly greater than progesterone while the ORG 2058 analog bound with only 7% of the affinity of the endogenous ligand. Radiolabeling was achieved via the corresponding tributylstannyl precursor in good yields and high radiochemical purity. *In vivo* tissue distribution studies were disappointing for all of the ligands. Only the Z-isomer of the iodovinyl ORG-3236 analog possessed selectivity for the progesterone rich tissues in normal female rat. However, this selectivity was not observed in the induced mammary tumors.

Although the studies focused on imaging, the failure to be retained by the target tissues would also be of concern for radiotherapeutic applications as well.

Reevaluation of the work of Katzenellenbogen may provide additional possibilities for radioiodinated analogs of progesterone receptor ligands (Fig. **7**). In particular the work with the 16α, 17α-dioxolanes provides opportunities to synthesize the corresponding iodinated analogs of the fluorinated compounds [26-28]. Conversion to the corresponding tributylstannyl derivatives followed by radioiododestannylation should yield target radiochemicals for *in vitro* and *in vivo* evaluation. Whether such products would overcome the deficiencies seen with previous agents, i.e., reduced affinity high nonspecific binding or metabolic lability, remains to be seen. A novel variation which would be amenable to the incorporation of a Auger-emitting metal ion has also been reported by this group [29].

## C. Androgen Receptor Ligands

Many of the same limitations imposed on progesterone receptor-directed ligands are encountered in the chemistry of the androgen receptor targeted agents. The endogenous ligands, testosterone and 5α-dihydrotestosterone have receptor affinities an order of magnitude less than that observed for estradiol at the target site. While there is an extensive literature related to androgenic and anabolic steroids, few of those compounds have higher affinities than 5α-DHT for the target receptor. In addition, the endogenous ligands are rapidly metabolized to products with much lower receptor affinities. As a result, very few compounds have been described which have high affinity, metabolic stability and the potential for incorporation of a radionuclide possessing the desired properties.

The work with radiolabeled androgenic steroids over the past 10 years has concentrated primarily on their radiodiagnostic (PET and SPECT) potential. This mostly represented an extension of studies conducted during the late 1980's in which radiohalogens I-125 or F-18 were incorporated at the 7α, 16α, or 17α-positions (Fig. **8**) [30-33]. These early results were generally disappointing in that the radiochemicals exhibited either little specific binding or metabolic lability, or both. The challenges, therefore, were to improve the receptor affinity and the stability of the C-I bond.

Hochberg and co-workers extended their studies of the 17α-[$^{125}$I]-iodovinyl testosterone and nortestosterone radioligands with the preparation of E- and Z-17α-iodovinyl-7α-methyl nortestosterone. The E-isomer was twice as potent as the Z-isomer but still less than 5α-DHT (RBA = 12 vs. 53, R1881 = 100 in rat cytosol). Unfortunately, when evaluated by Ali, *et al.*, the agent demonstrated little selectivity *in vivo* [23,34]. As a result, this compound was not examined for its ability to cause radiation induced cell death. Hochberg's group subsequently prepared a series of 7α-iodo (and fluoro) androgens as potential imaging agents. From this series, the radiohalogen was introduced by simple nucleophilic displacement into a steroid nucleus bearing appropriate 19/17α substituents [35,36]. They evaluated the effects of dihydro testosterone vs. dihydro nortestosterone

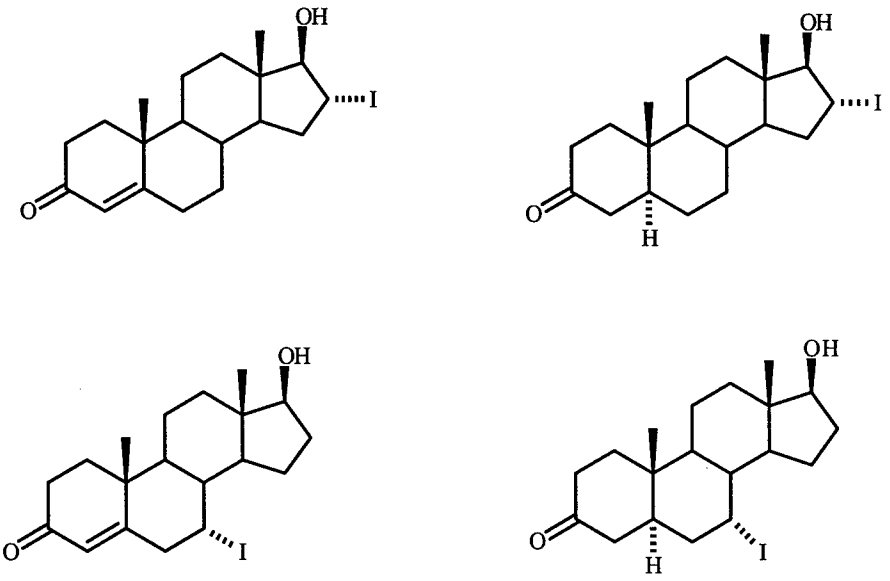


**Fig. (8)**. radioiodinated (dihydro)testosterone derivatives.

vs. 17α-methyl dihydro(nor)testosterone. While the affinities compared to 5α-DHT were quite good (RBA = 25-123, DHT = 100) the radioiodinated agents were ineffective both *in vitro* and *in vivo*. As a result, no further work was pursued with those radiochemicals.

Radiolabeled antiandrogens constitute an even smaller series of potential therapeutic agents. This is due in part to the relatively small number of compounds that display this type of pharmacological activity. Until recently only flutamide, anandron and bicalutamide were the only agents approved as antiandrogens although newer nonsteroidal compounds are in clinical trials. (Fig. **9**). Miller and coworkers [37] reported the synthesis of radioiodinated bicalutamide via the triazene and trimethyltin intermediates. The iodinated derivative had affinity greater than the parent compound (3.1 nM vs. 11.0 nM), however, this was still poorer than testosterone (1.1 nM). In their I-125/123 labeled form this radiochemical may have potential as a radiotherapeutic agent, but no further data has been provided since the initial disclosures.

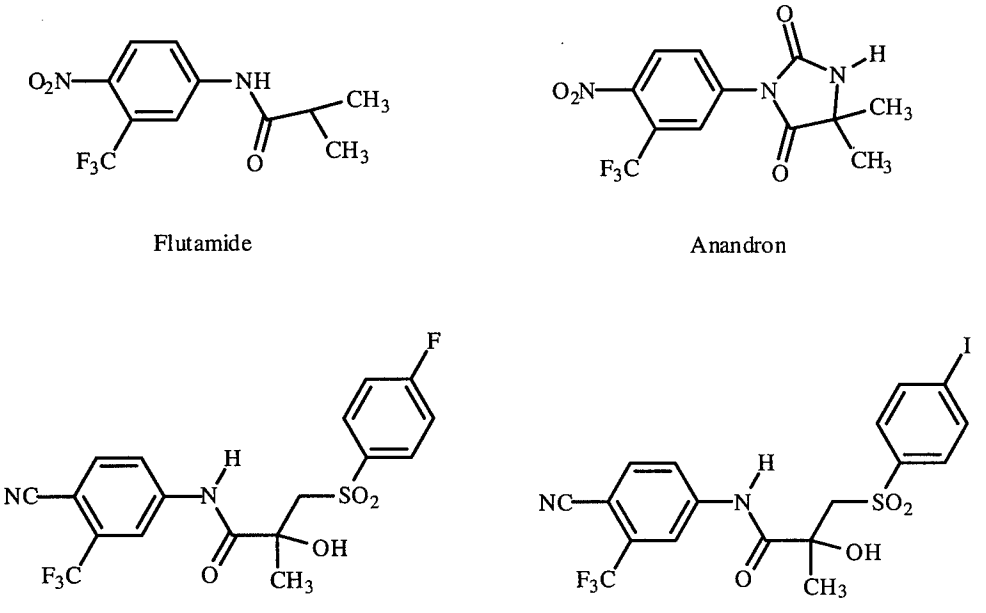


**Fig. (9)**. Antiandrogens and radioiodinated analog.

## D. Summary

The past decade has seen advances in the synthesis of Auger-emitting ligands, both agonists and antagonists, for the steroid hormone receptors. Strategies have been developed for maintaining substantial affinity for the receptor and imparting metabolic stability in most cases. Use of the radioiododestannylation has been the most successful means for rapidity incorporating the radiohalogen in high specific activity. So far only the estrogen receptor-directed agents have demonstrated the ability to produce significant tumor cell killing. Successful extension to therapy remains to be shown for the estrogenic ligands. Improvements in receptor affinity and metabolic stability are required before the progesterone and androgen receptor directed agents can be evaluated as therapeutic agents.

## III. DNA DIRECTED AGENTS

This section examines the work done over the past 10 years to develop agents that directly target the DNA. Deoxyribonucleosides (D nucleotides) and DNA intercalating agents constitute two other classes of compounds capable of imparting the cytotoxic effects of Auger-emitting radionuclides to the nuclear DNA. Labeled analogs of the deoxyribonucleotides can be incorporated into the DNA by the enzyme DNA polymerase if they resemble the endogenous substrate. This is one of the mechanisms by which antineoplastic drugs such as 6-mercaptopurine, 6-thioguanine, and adenine arabinoside, exert their cytotoxic effects. Appropriate nucleosides containing iodine or bromine could also be incorporated into the DNA and, upon disintegration, provide the low energy electron shower directly onto the DNA. Intercalating agents, on the other hand, are polycyclic compounds of either natural or synthetic origin that insert themselves between the bases of the DNA. Their ability to disrupt or to stabilize the structure of the DNA inhibits processes associated with DNA replication and ultimately exerts a cytotoxic affect. Auger-emitting analogs of the intercalating agents have the ability to induce strand breaks if the nuclear decay occurs during the time that the agent resides in the helix.

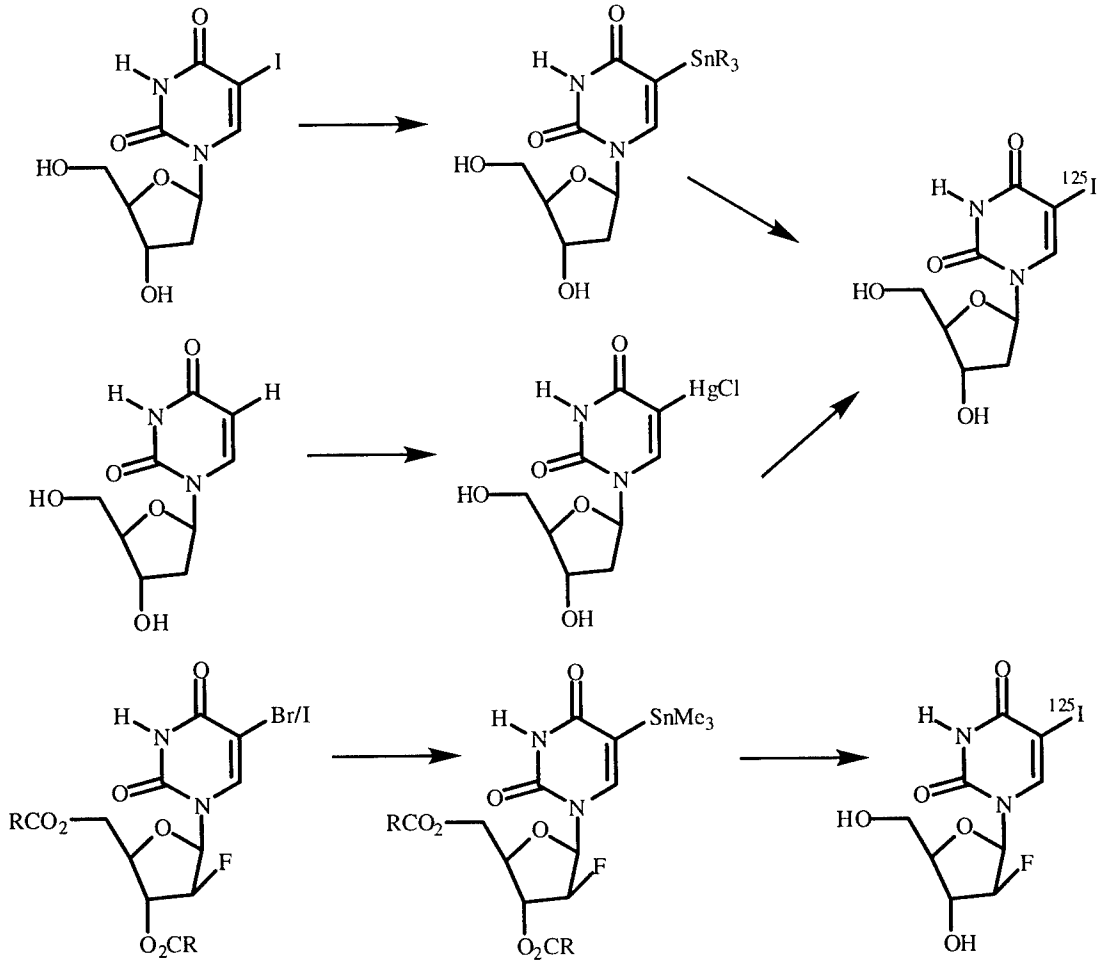


**Fig. (10).** representative syntheses of radioiodinated nucleosides.

## A. Radiolabeled Nucleoside Analogs

Among the nucleosides which could be applied to radiotherapy of tumors, halogenated analogs of uracil have been most extensively evaluated. This emphasis is the result of earlier studies that suggested that 5-iodouracil in particular is a close structural analog of thymidine and that it substitutes for the natural pyrimidine base in many of the ribosylation and kinase reactions preceding incorporation into DNA. The two major strategies are the synthesis of the radiohalogenated derivatives that incorporate improvements in the radiohalogenation procedure itself and the synthesis of nucleosides with improved biological characteristics.

Among the examples of radiohalogenations of nucleosides or their derivatives, two that best illustrate the methodological improvements are the synthesis of iododeoxyuridine and its 2-deoxy-2-fluoro analog (Fig. **10**). The preparation of the former agent was reduced to a kit formulation by Foulon and Kassis [38,39]. In one method, they chloromercurated deoxyuridine to give the 5-chloromercuri-derivative which could be converted to the radioiodinated product using labeled iodide and Iodogen. The alternate procedure began with the cold iododeoxyuridine which was converted to the 5-trialkylstannyl intermediate with Pd(0) catalyst and hexaalkylditin. Radioiodination with iodide and hydrogen peroxide then gave the desired product. Both methods were virtually instantaneous, however, the demercuration method was more applicable to kit use. Vaidyanathan and Zalutsky [40] also employed the stannylation-destannylation method, however, their brominated or iodinated precursor required synthesis from the arabinoside and pyrimidine starting materials. The key iododestannylation step proceeded in greater than 85% yields to give the desired products.

The preparation of novel nucleosides/nucleotides is illustrated by two recent examples (Fig. **11**). Dougan,*et al.*, [41] began with iododeoxyuridine and following protection as the 5-Fmoc ester coupled it at the 5-position of the pyrimidine with bis(tributylstannyl)ethylene. Activation at the 3-position of the sugar with a phosphoramidate group allowed the intermediate to be incorporated into an oligonucleotide that was ultimately radioiodinated using [I-125]-iodide and various oxidants. Reed, *et al.*, [42] also prepared a radioiodinated oligonuleotide via iododestannylation . In their synthesis, however, they utilized a sequence that contained a terminal hexamethyleneamine to which a 4-tributylstannylbenzoyl moiety could be conjugated. Radioiodination used their standard method and the product was obtained in good yields and high purity. Although the investigators implied potential radiotherapeutic applications, no data were provided.

## B. DNA Minor Groove Binding Agents

Another approach for the design of Auger-emitting DNA targeted agents involves labeling

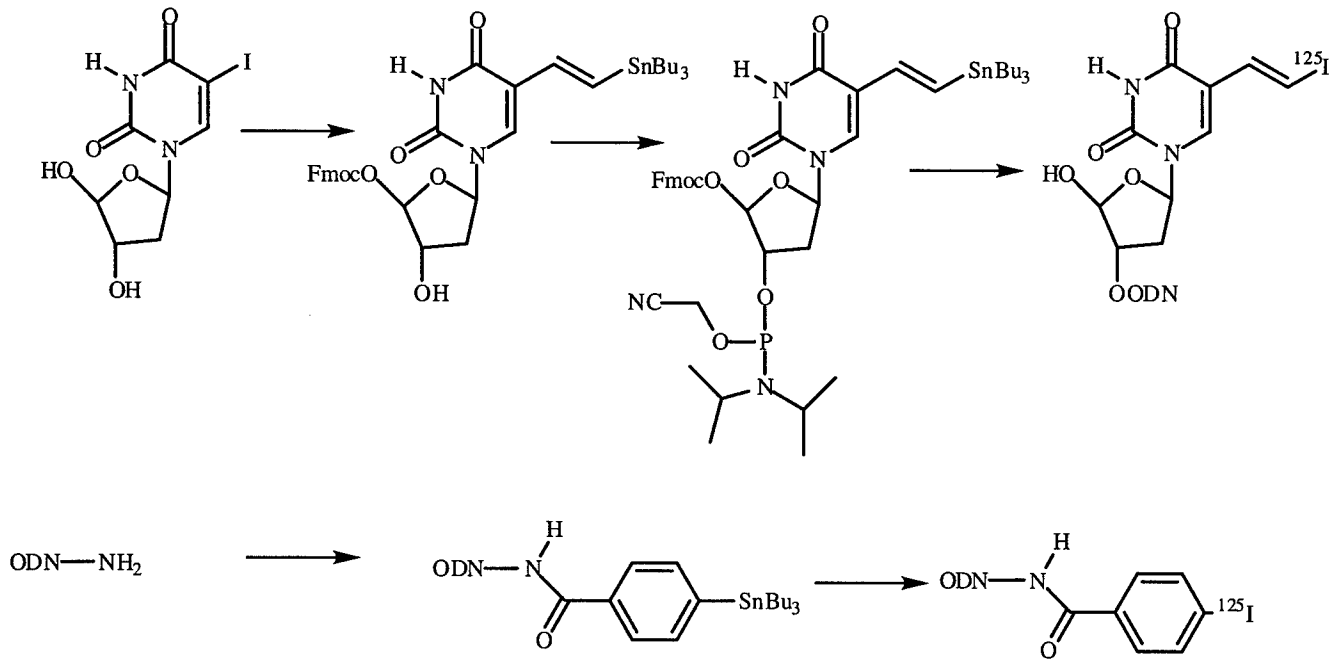


**Fig. (11).** Examples of Radioiodinated oligonucleotides.

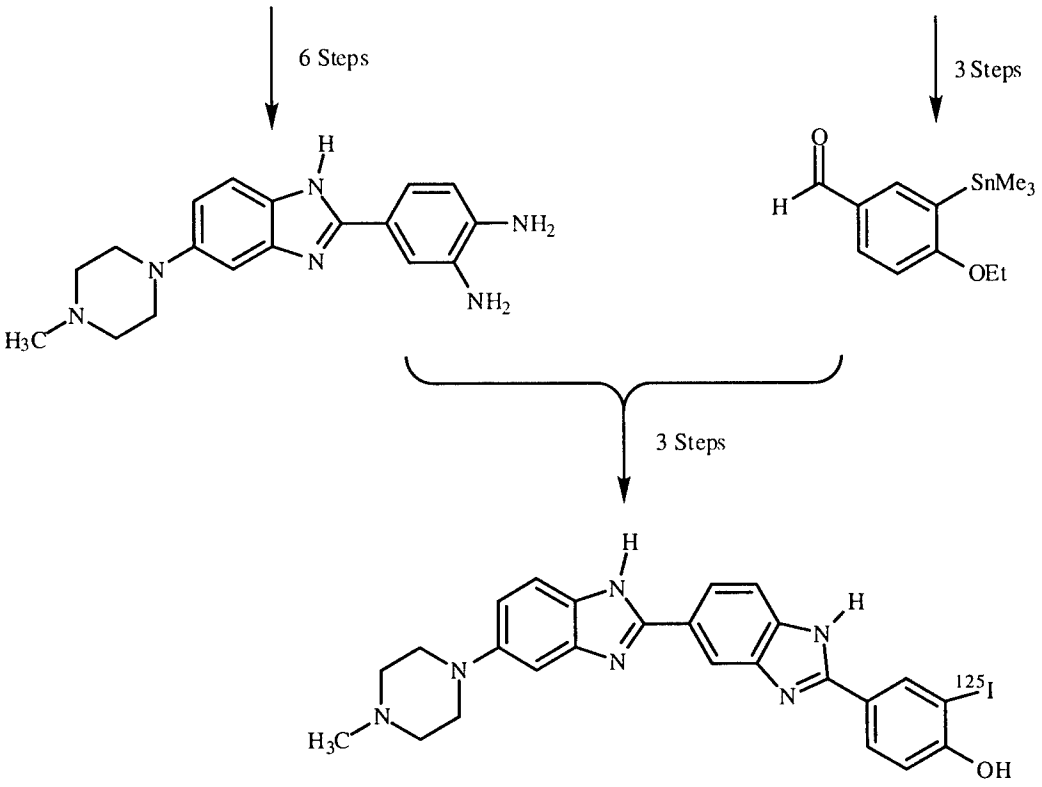


**Fig. (12).** Synthesis of radioiodinated iodoHoechst 33342.

compounds that bind to the minor groove of the DNA via multiple hydrogen bonds. An example of the labeled intercalator method is illustrated by the synthesis and evaluation of [I-125]-iodoHoechst 33342 by Kassis and co-workers [43-46]. In their synthesis (Fig. **12**), it was necessary to choose a site which could simultaneously permit the insertion of the trimethylstannyl group for radiolabeling while not adversely affecting the binding of the agent to the DNA. This was achieved by inserting an iodine on the distal aryl ring that could be replaced by the requisite stannyl moiety. With the availability of other sequence selective minor groove binding agents related to netropsin and distamycin [47] it should be possible to prepare and evaluate other Auger-emitting compounds as therapeutic agents. A relevant example is the modification of a sequence selective binder by Sigurdsson [48] to crosslink DNA. Replacement of the alkylating group by a labeled conjugate may achieve a comparable biological effect.

## C. Summary

In the area of DNA targeted agents there has been modest progress in the field of radiosynthesis. While methods have been developed for the efficient preparation of labeled nucleosides, both for incorporation into DNA or into oligonucleotides that bind to the DNA, it is not clear whether the *in vivo* incorporation of the agents is sufficient to induce effective cytotoxicity. A similar problem may exist with the minor groove binding agents, however, the flexibility in their construction may ultimately lead to diagnostic or therapeutic agents.

## IV OTHER SYNTHESIS OF AUGER ELECTRON-EMITTING AGENTS

Although the majority of radiosynthesis of (potentially) Auger electron-emitting agents have focused on the nuclear DNA as their ultimate target, studies on other approaches have also been reported. Radioiodinated antibodies with anticancer potential continue to be evaluated, with the utilization of Auger electrons perhaps as part of their mechanism of action. While most radioiodinations use the conventional electrophilic incorporation with an oxidant [49,50], others use the trialkylstannylaryl carboxylate NHS ester conjugating agent [51]. This latter procedure continues to generate interest, not only for its diagnostic potential but also for incorporating Auger electron-emitting radionuclides [52-56]. Since there have been some studies exploring the utility of Auger emissions as a therapeutic adjunct

in the MIBG treatment of neuroblastoma [57,58] syntheses of other radiolabeled MIBG analogs have been reported [59]. Whether this is a viable approach to therapy remains to be seen. Lastly, the preparation of a somatostatin analog containing a chelated Auger electron-emitting radionuclide was described by Heppeler, *et al.* [60]. While little biological data were provided, its synthesis constituted one of the very few instances that did not employ a radiohalogen.

## NOTE ADDED IN PROOF

Since the submission of the review, three relevant manuscripts have been published. The first article involved the preparation and evaluation of new nonsteroidal antiandrogens related to bicalutamide ( Kirkovsky, *et al., J. Med. Chem.* **2000**, *43*, 581-590). The second paper evaluated the binding of iodinated Hoechst 33258, a structural analog of the DNA intercalator prepared by Kassis (Squire, *et al., Nucl. Acids Res.* **2000**, *28*, 1251-1258). The third paper described the production of In-114m, an Auger-emitting radionuclide, and the subsequent preparation of [In-114m]-DTPA-D-Phe-octreotide (*Nucl. Med. Biol.* **2000**, *27*, 183-188).

## ACKNOWLEDGMENTS

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# Conformational Studies of Novel Estrogen Receptor Ligands by 1D and 2D NMR Spectroscopy and Computational Methods

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**Abstract:** The solution conformations of the novel estrogen receptor ligands, (17α, 20E)-(p-α, α, α-trifluoromethylphenyl)vinyl estradiol (**1**) and (17α, 20 E)-(o-α, α, α-trifluoromethylphenyl)vinyl estradiol (**2**) were investigated in 2D and 1D NOESY studies and by comparison of $^{13}C$ NMR chemical shifts with theoretical shieldings. The $^{1}H$ and $^{13}C$ assignments of **1** and **2** were determined by DEPT, COSY, and HMQC experiments. The conformations of the 17α-phenylvinyl substituents of **1** and **2** are of interest because of their differing receptor binding affinities and effects in the *in vivo* uterotrophic growth assays. A statistical method of evaluating contributing conformers of **1** and **2** from predicted $^{13}C$ shifts of possible structures correlated quite well with conformational conclusions derived from the NOE data. The 17α substituents of **1** and **2** apparently exist in similar conformational equilibria, suggesting that while **1** and **2** would occupy a similar receptor volume, interactions with the protein may shift the equilibrium and thereby influence the expression of the ligand.

## Introduction

As part of our efforts to develop more effective therapeutic agents for the treatment of breast cancer, we undertook the designing of (17α, 20E)-X-phenylvinyl estradiol compounds that can potently and selectively modify the interaction of estradiol with its target receptor to impart the desired biological effect. In the binding assay of (17α,20E)-(p-α,α,α-trifluoromethylphenyl)vinyl estradiol (**1**) and (17α,20E)-(o-α,α,α-trifluoromethylphenyl)vinyl estradiol (**2**) with the estrogen receptor ligand binding domain (ER-LBD) **2** had a relative binding affinity (RBA) of 223 at 25°C compared to RBA=8 for **1**. This difference in potency was also expressed *in vivo* in the rat uterotrophic growth assay where **2** had an EC50 = 0.31 nmoles compared to the EC50 = 10.6 nmoles for **1**.[1] Because the 17α-phenylvinyl substituent may interact with the key helix-12 of the ligand binding domain (LBD) of the estrogen receptor (ER)[1], we considered that differences in the preferred conformation of **1** and **2** could account for their distinguishable biological responses and varying binding affinity. (Insert Structure)

Recently we showed that the placement of a substituent in the *ortho* or *para* position of (17α, 20 Z)-phenylvinyl estradiol affected the conformational equilibrium of the 17α-side chain.[2] In that study, (17α, 20Z)-(p-methoxyphenyl)vinyl estradiol and (17α, 20 Z)-(o-α,α,α-trifluoromethylphenyl)vinyl estradiol compound were found to exist in similar conformational equilibria which suggested they would likely occupy a similar receptor volume. These results were consistent with their similar RBA values of 20 and 23. In contrast, (17α, 20 Z)-(o-hydroxymethylphenyl)vinyl estradiol, which had an RBA of 140, was found to exist in a different conformational equilibrium. These results

suggested that in addition to position and electronic effects of the substituent, the conformational equilibria of the 17α substituent of Z-compounds may account for the varying RBA values.

In this report, we present a conformational study of **1** and **2** using NMR and computational methods, to determine whether differences in the preferred conformation of **1** and **2** may also account for their distinguishable biological responses and binding affinity.

The key conformational feature to establish for **1** and **2** is the orientation of the 17α substituent relative to the steroid skeleton. In this study, we use molecular mechanics calculations to generate a set of possible conformations. Two types of NMR data are used in conjunction with the predicted conformations to evaluate which conformations are populated in solution. One approach is to use $^{13}C$ chemical shifts in a comparison with shifts predicted for each of the geometries generated from the molecular mechanics calculations. The predicted $^{13}C$ shifts come from empirically scaled GIAO (gauge including atomic orbitals) shielding calculations.[3] The other approach is to compare $^{1}H$-$^{1}H$ nuclear Overhauser effects established in one- and two- dimensional experiments, 1D and 2D NOESY, with predicted interatomic distances.

## Experimental

The syntheses and biological data of compounds **1** and **2** have been described elsewhere.[14] $^{1}H$ NMR data were recorded at 25°C for 5-8 mg samples dissolved in acetone-$d_6$ in 5 mm NMR tubes using a Varian Unity 500 MHz NMR spectrometer equipped with a 5 mm Varian inverse probe. DEPT and $^{13}C$ experiments were obtained on a Varian Mercury instrument at 75 MHz.

$^1$H spectra were obtained with a spectral width (SW) of 8 kHz, a 67° pulse flip angle, a 1.7 s acquisition time (AT), a 2 s relaxation delay (RD) and digitized with 32768 points giving a digital resolution (DR) of 0.488 HZ per point. Chemical shifts were referenced to the residual $^1$H signal of acetone-$d_6$.

$^1$H-decoupled $^{13}$C spectra were recorded with a 18856 SW, a 60° pulse flip angle, a 2 s RD and digitized into 65536 points to give a digital resolution of 0.575 Hz per point.

HMQC[5] experiments for single bond $^1$H, $^{13}$C chemical shift correlation spectra utilized the BIRD sequence to suppress unwanted signals and GARP[6] $^{13}$C decoupling. Two sets of 256 time increments were obtained in the phase-sensitive mode with 32 transients obtained per time increment and a 2 s RD. The final matrix was processed with Gaussian functions.

COSY45[7] experiments were performed with 8 scans for each of 200 increments in $F_1$, 2048 data points in $F_2$ and a relaxation delay of 2.0 s. The final matrix was symmetrized and processed with sine-bell exponential multiplication.

NOESY[8] experiments were performed with 32 scans for each of 256 $F_1$ increments, 2048 data points in $F_2$, with a relaxation delay of 2.0 s and a mixing time of 0.500 ms. The final matrix was not symmetrized, but was processed with Gaussian weighing functions.

1D NOESY[9] spectra were obtained using a spectral width of 5000 Hz and 20500 points giving a digital resolution of 0.490 Hz per point, a mixing time of 0.500 ms, a RD of 2.0 s, and a AT of 1.7 s. A Gaussian shaped pulse was used for selective irradiation.

## RESULTS AND DISCUSSION

### $^1$H and $^{13}$C Assignments

The $^1$H NMR spectra of **1** and **2** in acetone-$d_6$ (Figures 1(a) and 2(a)) exhibit very little chemical shift dispersion in the low frequency spectral regions (1.2-2.5 ppm) even at 500MHz, precluding straightforward $^1$H assignment. However, $^1$H signals were assigned via application of HMQC and COSY techniques. Starting with the $^{13}$C shift assignments that were based on our earlier studies of several 17$\alpha$-substituted estradiols, DEPT experiments, and theoretical shielding calculations (see below), geminal proton resonances were identified and all proton signals were correlated with directly attached carbons via an HMQC experiment. COSY experiments confirmed the initial assignments made by the HMQC experiment but did not, of course, distinguish between $\alpha$ and $\beta$ hydrogens in a given methylene group. This distinction was readily achieved by 1D NOESY experiments (Figure 1(b) and 2(b)). Using a Gaussian pulse, selective irradiation of the protons of the methyl group enhances protons on the $\beta$ face of the C and D rings, viz., 11$\beta$, 12$\beta$, 15$\beta$, 16$\beta$, and H8. Table 1 lists the complete assignments of the $^1$H and $^{13}$C signals of **1** and **2**.

### Theoretical Carbon Chemical Shifts and Conformational Determination

The predicted low energy conformers of **1** and **2** (Figures 3 and 4) were generated using the MM3[10] force field through conformational searching by previously described method.[3] The key dihedral angles are listed in Table 2 for the lowest energy conformers, **1a-1c** and **2a-2h**, with energies within 1.5 kcal of the lowest energy conformers for **1** and

**2**, respectively.

As the MM3 calculations show, significant changes in the 17α side chain conformation result in only minor energy differences. Most of the low energy conformers are within 1 kcal of the lowest energy conformer, making any conformational determination based purely on MM3 energy predictions unreliable. In MMX[11] and MM3 force fields, driving the dihedral angle C21-C20-C17-C13 shows a very shallow energy surface from 85° to 165° (Figure 5). In this region, discrete changes in the orientation of the phenyl to the vinyl group yielded numerous minima using either MM3 or MMX. Conformers **1a** and **1c** were kept as minima since they represent the upper and lower dihedral range of this shallow surface.

More reliable conclusions regarding the preferred 17α side chain conformation of **1** and **2** could be achieved by applying a statistical method of determining contributing conformers from predicted $^{13}C$ chemical shifts, $\delta_{pred}$, of MM3 determined conformers.[12] These $\delta_{pred}$ were calculated by empirically scaling GIAO-calculated absolute shieldings[4], σ, obtained at the B3LYP/3-21G level with heteroatoms augmented at the 6-31+G* level. All shielding calculations were carried out with the Gaussian98 program.[13] Tables 3 and 4 list the $\delta_{pred}$ of each MM3 conformer and the assigned experimental $^{13}C$ chemical shifts, $\delta_{exp}$.

In this statistical method, the predicted $^{13}C$ shifts of the C and D rings of all MM3 conformers of **1** and **2** were in each separate case treated as independent variables in a multiple independent variable regression analysis of the corresponding experimental data.[14] The predicted $^{13}C$ shifts of the A and B rings of all reasonable conformers of **1** and

**2** were not used in this statistical analysis since most are within 1 ppm of the experimental values regardless of the conformer. In contrast, most carbons in the C and D rings of **1** and **2** displayed significant shift differences depending on the geometry. The regression analysis yielded fractional populations as the fitting parameters. All standard errors and confidence levels of the regression analysis were estimated using the Bootstrapping method.[15]

The results and corresponding estimates of uncertainties (standard errors) are listed in Table 5. Both **1** and **2** were found to have a major conformer, **1b** 72(32)% and **2e** 65(33%). Minor conformers are also indicated for each: **1a** 13(29%) and **1c** 15(28%), and **2c** 33(18%) and **2h** 2(22%). It is important to note that the large corresponding standard error of certain contributing conformers, such as for minor conformers **1a**, **1c**, and **2h,** makes conclusions on their presence unreliable.

## NOESY Studies

The solution state conformations of the 17$\alpha$ side chain of **1** and **2** were also investigated by measuring NOE intensities between the vinyl protons and the aliphatic $^1$H of the C and D ring. The 2D NOESY of **1** and **2** reveal a similar pattern of NOE cross peaks and intensities between H20 and H21 with the aliphatic protons 12$\alpha$, 12$\beta$, H14, 15$\alpha$, 16$\alpha$, and 16$\beta$ (Figure 6). Selective 1D NOESY experiments of H20 and H21 provided a more detailed inspection of the NOE intensities (Figure 1(c) and 2(c), (d)). Table 6 and 7 summarize and compare the intensities of the observed NOE signals with expected NOE's based on H-H distances in all predicted low energy conformers of **1** and **2**.

The NOE data of **1** and **2** suggest a similarly preferred orientation of the 17α side chain. The absence of an observable NOE between H21 and 12α rules out conformers, **1c**, **2a**, **2b**, **2c**, **2g**, and **2h**, as contributing conformers, precluding most of the minima observed in the shallow energy surface range of 85° to 165° for dihedral C21-C20-C17-C13 . The observable NOE's between H21 and H14, 15α, and 16α are consistent with **1b** and **2e**. The presence of the extended conformers, **1a**, **2d**, and **2f** are evident from the weak enhancements of 15α and 16α upon irradiation of H20.

The NOE data of **1** and **2** and the statistical analysis of $^{13}C$ chemical shifts are both consistent with a preferred orientation of the 17α side chain. The findings from the multiple independent variable linear regression analysis of the $^{13}C$ data of **1** and **2**, that conformers **1b** and **2e** are 72% and 65% populated, are compatible with the identity of the major conformer favored by NOE data. For **1**, the regression analysis predicts a 12% population of the extended conformer **1a,** which is also consistent with the NOE data. The inclusion in the statistical analysis of a 15% population of **1a** and a 33% population of **2c** is inconsistent with NOE data. This limitation in the regression analysis can be explained by the small $^{13}C$ shift differences in the C and D ring among most of the MM3 predicted conformers. However, the ability of the regression, based on predicted $^{13}C$ shifts to identify the same conformer among a competing set of conformers, as suggested by NOE data, demonstrates that this approach to interpretation of chemical shift data is a powerful complement to more common methods of conformational analysis.

## Conclusions

This study reveals that the 17α substituent of **1** and **2** have a similarly preferred

orientation in reference to the steroidal skeleton. The similarity in solution conformations of **1** and **2** suggests that they may occupy a similar receptor volume. Thus, other influences such as position and electronic effects of the substituent and their interactions with the complementary protein residues may also play roles in the differing biological responses and RBA values of **1** and **2**. It is interesting to note the apparent absence of a similar range of conformers among **1** and **2**, despite the predictions of favorable energies in MM3 calculations.

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## Figure Captions

**Figure 1.** (a) Low frequency spectral region of the 500 MHz $^1$H NMR spectra of **1** in acetone-$d_6$. Equivalent spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **1** obtained by selective irradiation of the C18 methyl (b), and H20/H21 (c) using a Gaussian pulse. Spectra (b) and (c) are 4x the vertical scale of (a). Overlap of H20 and H21 inhibited selective irradiation of each proton.

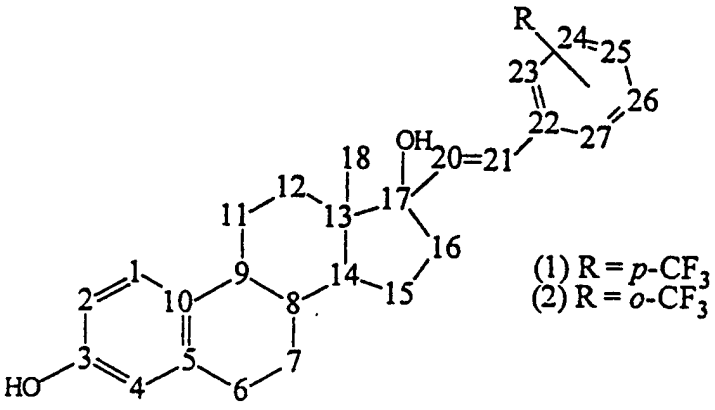
**Figure 2.** (a) Low frequency spectral region of the 500 MHz $^1$H NMR spectra of **2** in acetone-$d_6$. Equivalent spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **2** obtained by selective irradiation of the C18 methyl (b), H20 (c), and H21 (d) using a Gaussian pulse. Spectra (b), (c), and (d) are 4x the vertical scale of (a).

**Figure 3.** MM3-predicted geometries for the most stable conformers of **1**.

**Figure 4.** MM3-predicted geometries for the most stable conformers of **2**.

**Figure 5.** Dihedral driver of C21-C20-C17-C13 using (a) MMX and (b) MM3. Y axis represents increasing relative energy.

**Figure 6.** Spectral regions of the 500 MHz 2D NOESY spectrum of (a) **1** and (b) **2** obtained with a mixing time of 500 ms. The NOE connectivities are indicated.




(1) R = *p*-$CF_3$
(2) R = *o*-$CF_3$

**Figure Captions**

**Figure 1.** (a) Low frequency spectral region of the 500 MHz $^1$H NMR spectra of **1** in acetone-$d_6$. Equivalent spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **1** obtained by selective irradiation of the C18 methyl (b), and H20/H21 (c) using a Gaussian pulse. Spectra (b) and (c) are 4x the vertical scale of (a). Overlap of H20 and H21 inhibited selective irradiation of each proton.

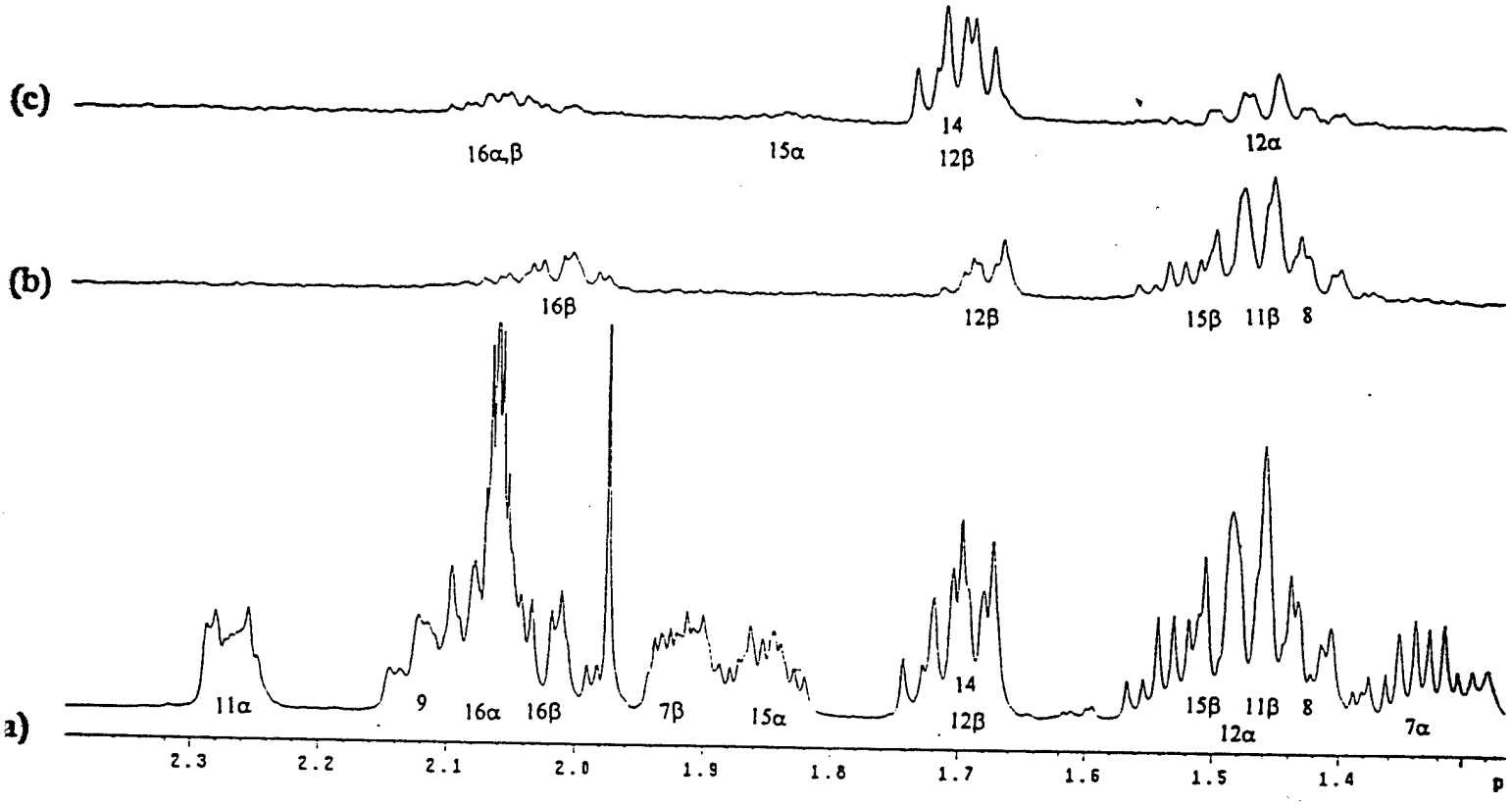
**Figure 2.** (a) Low frequency spectral region of the 500 MHz $^1$H NMR spectra of **2** in acetone-$d_6$. Equivaient spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **2** obtained by selective irradiation of the C18 methyl (b), H20 (c), and H21 (d) using a Gaussian pulse. Spectra (b), (c), and (d) are 4x the vertical scale of (a).

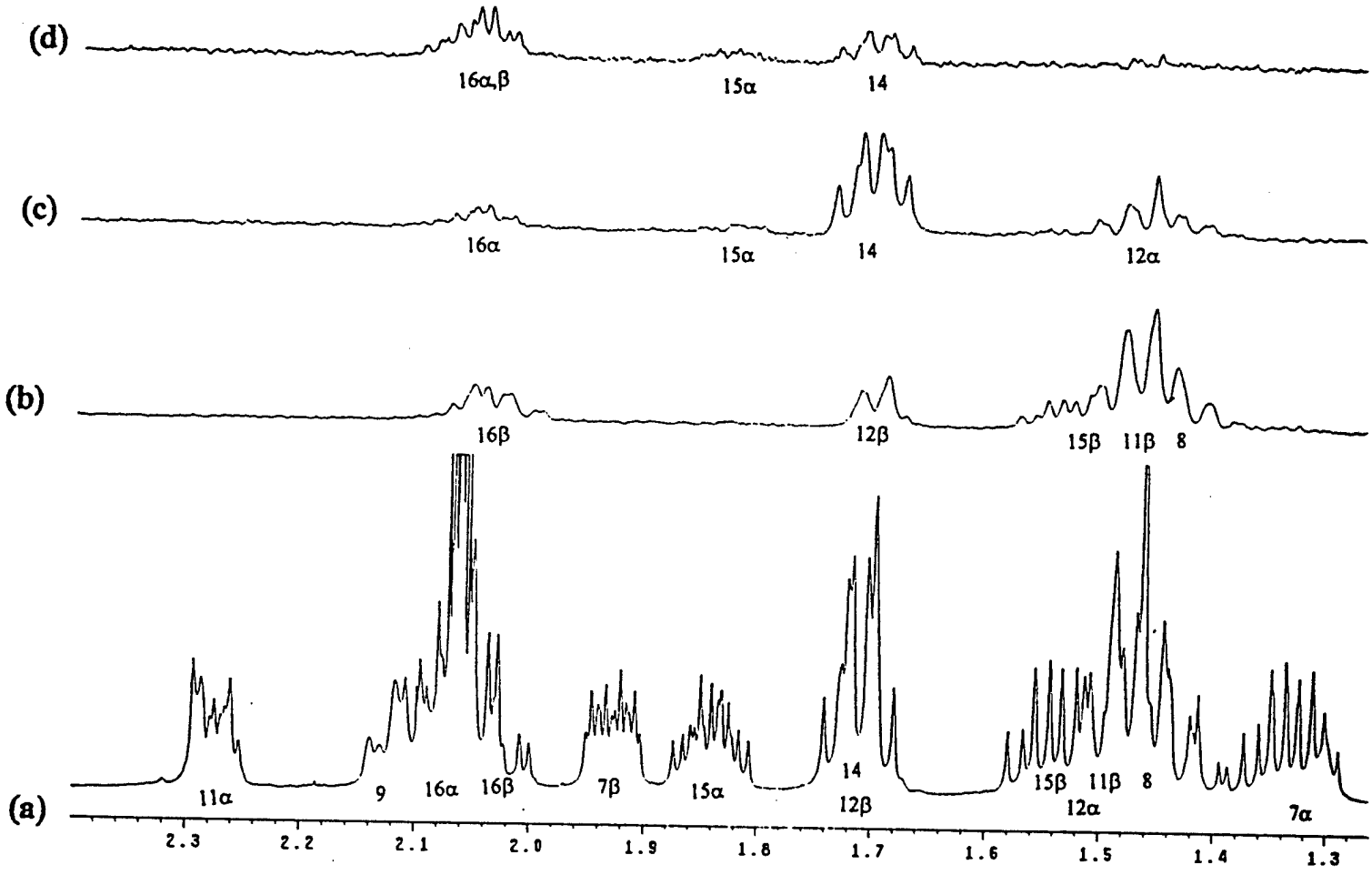
**Figure 3.** MM3-predicted geometries for the most stable conformers of **1**.

**Figure 4.** MM3-predicted geometries for the most stable conformers of **2**.

**Figure 5.** Dihedral driver of C21-C20-C17-C13 using (a) MMX and (b) MM3. Y axis represents increasing relative energy.

**Figure 6.** Spectral regions of the 500 MHz 2D NOESY spectrum of (a) **1** and (b) **2** obtained with a mixing time of 500 ms. The NOE connectivities are indicated.


(c)
16α,β
15α
14
12β
12α
(b)
16β
12β
15β 11β 8
a)
11α
9
16α 16β
7β
15α
14
12β
15β 11β 8
12α
7α
2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4
p


(d)
16α,β
15α
14
(c)
16α
15α
14
12α
(b)
16β
12β
15β
11β
8
(a)
11α
9
16α
16β
7β
15α
14
12β
15β
11β
8
12α
7α
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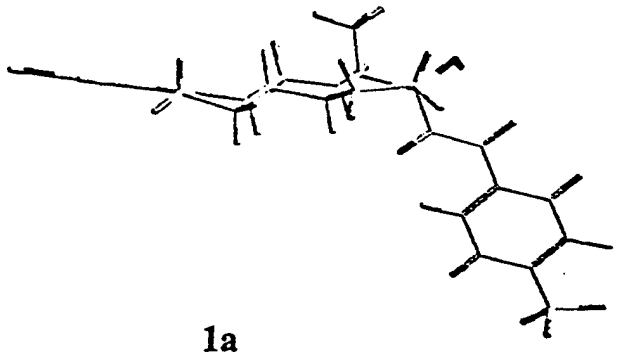
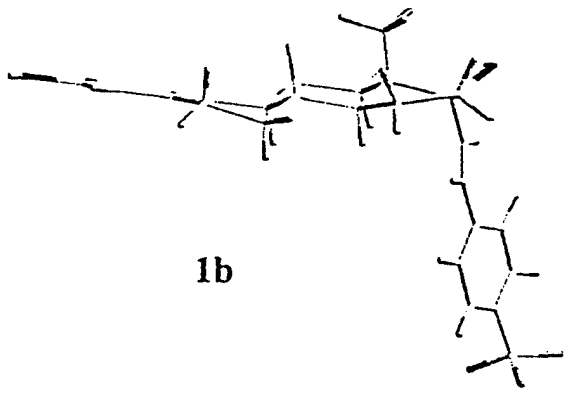
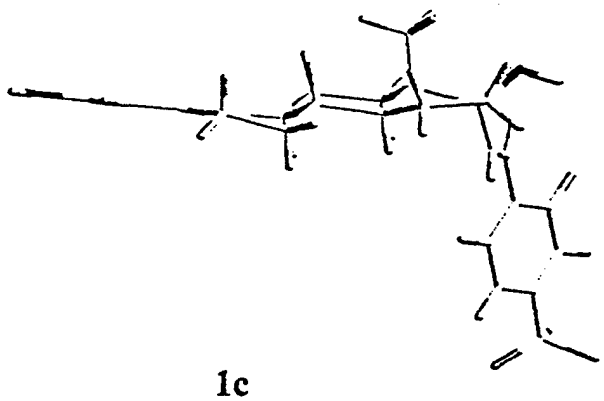
1.8
1.7
1.6
1.5
1.4
1.3



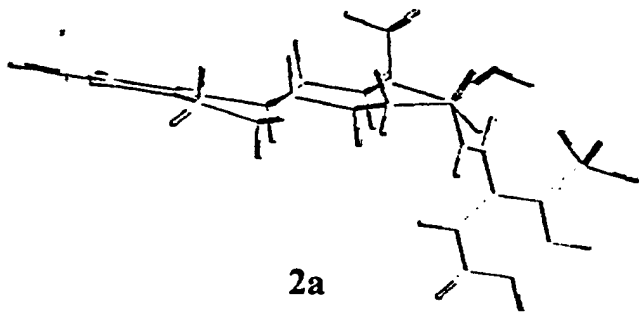
**1a**



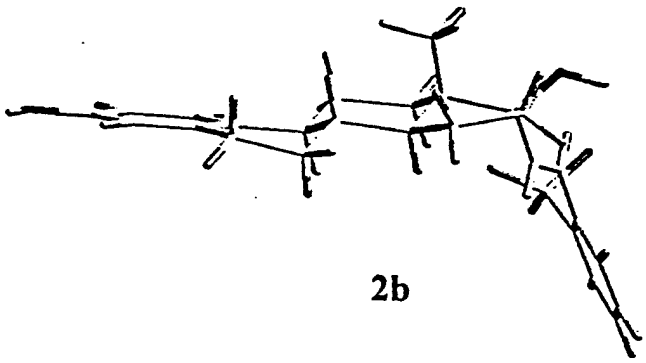
**1b**



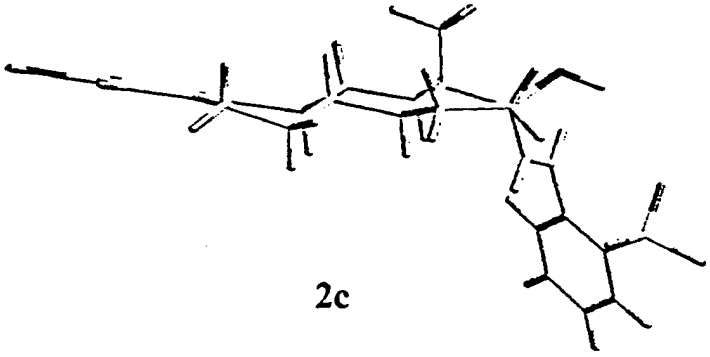
**1c**



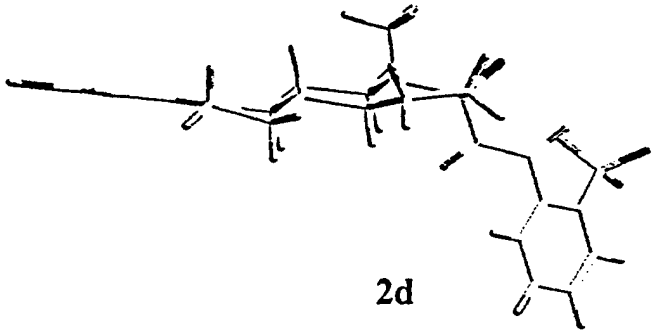
2a



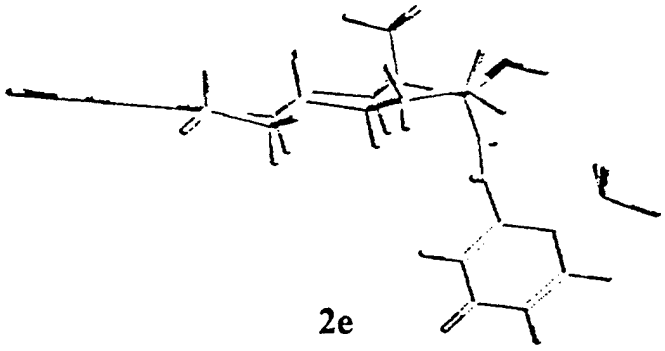
2b



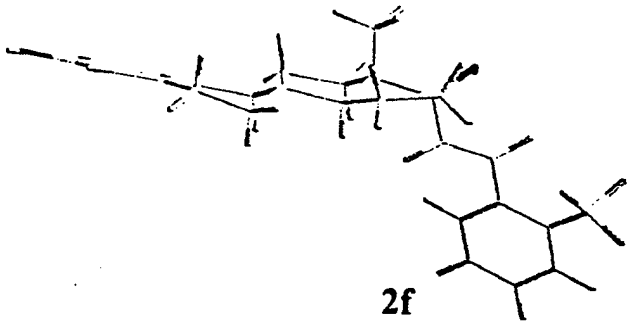
2c



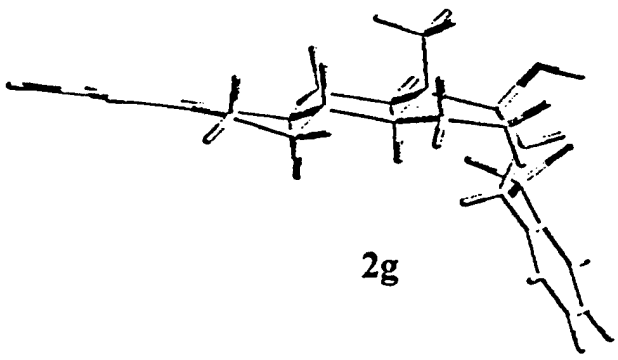
2d



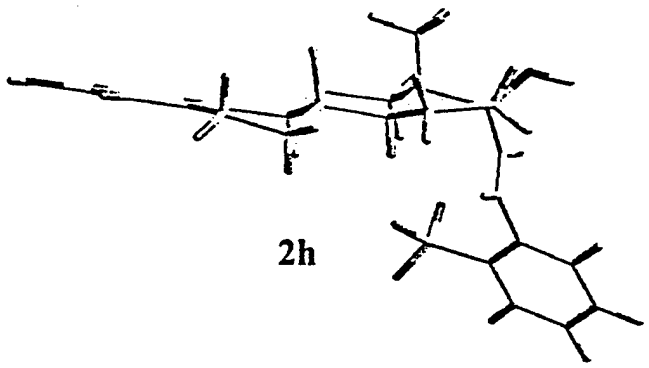
2e



2f

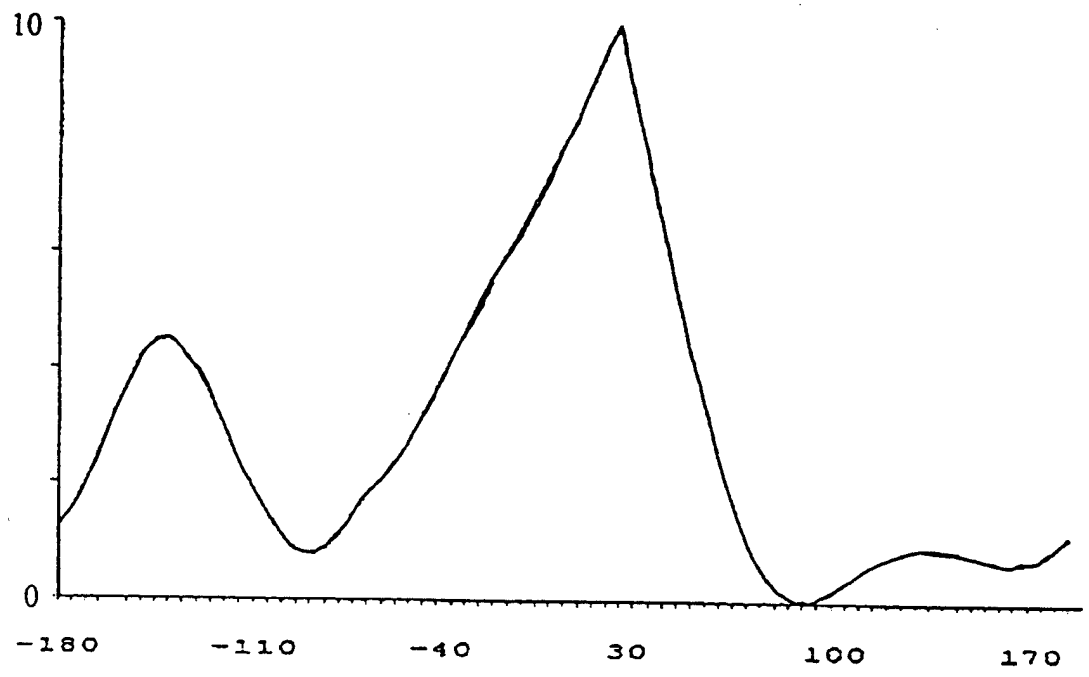


**2g**

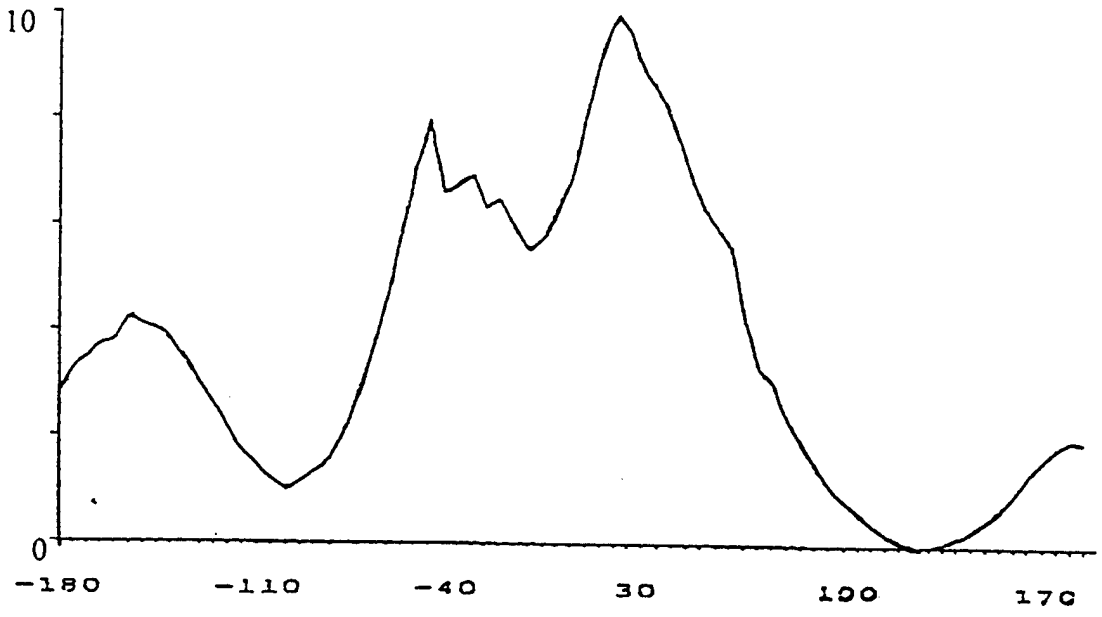


**2h**

(a)




(b)



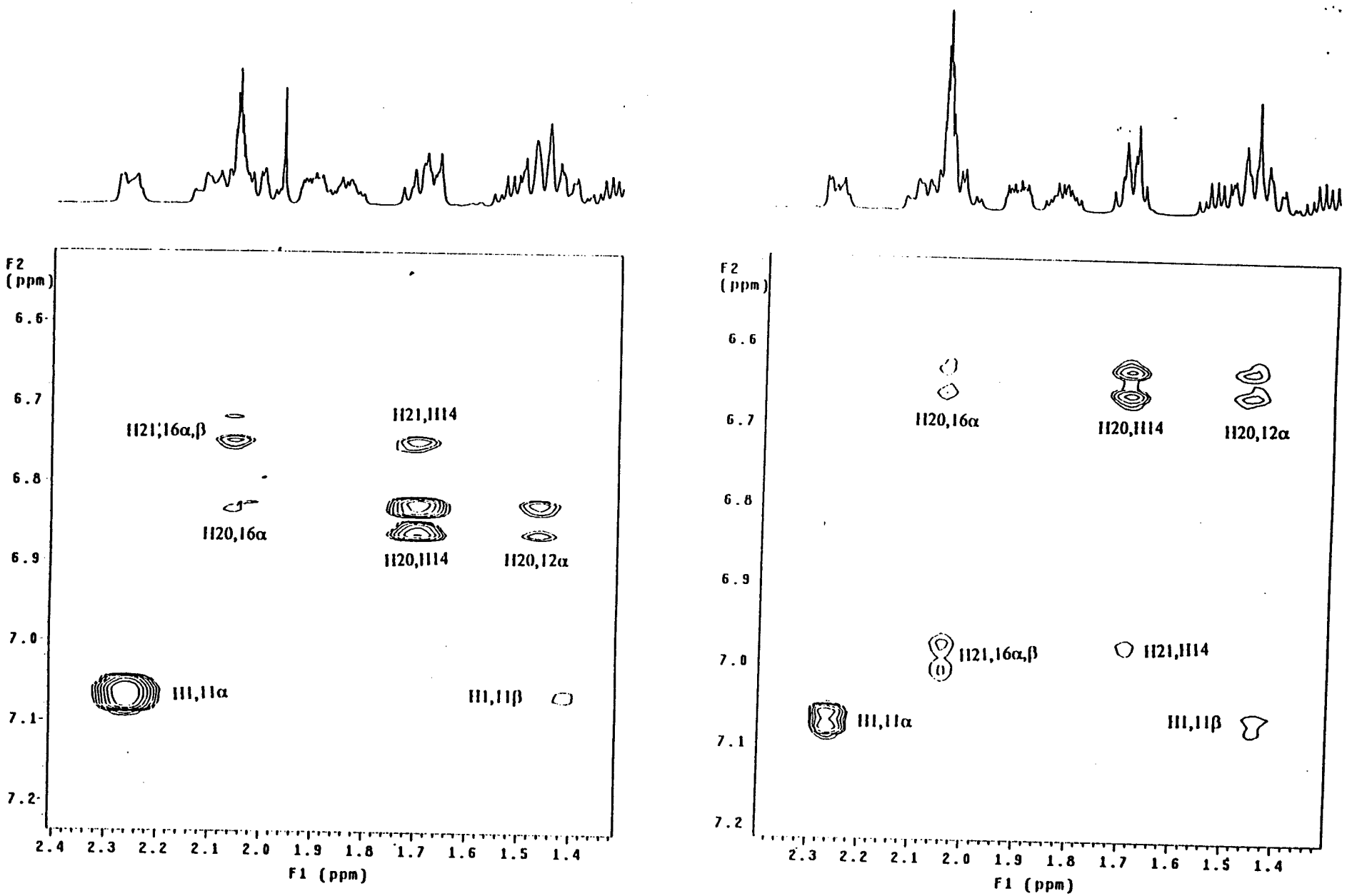

F2 (ppm)
6.6
6.7
6.8
6.9
7.0
7.1
7.2
H21,16α,β
H21,H14
H20,16α
H20,H14
H20,12α
H1,11α
H1,11β
2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4
F1 (ppm)
(a)
F2 (ppm)
6.6
6.7
6.8
6.9
7.0
7.1
7.2
H20,16α
H20,H14
H20,12α
H21,16α,β
H21,H14
H1,11α
H1,11β
2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4
F1 (ppm)
(b)

**Table 1.** $^{1}H$ and $^{13}C$ Chemical Shifts for **1** and **2**

| $^{1}H$ | 1 | 2 | $^{13}C$ | 1 | 2 |
|---|---|---|---|---|---|
| 1 | 7.09 | 7.09 | 1 | 126.4 | 126.4 |
| 2 | 6.58 | 6.59 | 2 | 112.9 | 112.9 |
| 4 | 6.52 | 6.54 | 3 | 155.3 | 155.2 |
| 6α | 2.75 | 2.78 | 4 | 115.3 | 115.3 |
| 6β | 2.80 | 2.81 | 5 | 137.7 | 137.5 |
| 7α | 1.32 | 1.34 | 6 | 30.0 | 29.9 |
| 7β | 1.92 | 1.92 | 7 | 27.7 | 27.7 |
| 8 | 1.43 | 1.54 | 8 | 40.1 | 40.0 |
| 9 | 2.10 | 2.10 | 9 | 44.0 | 44.0 |
| 11α | 2.26 | 2.28 | 10 | 131.3 | 131.3 |
| 11β | 1.46 | 1.43 | 11 | 26.7 | 26.6 |
| 12α | 1.42 | 1.50 | 12 | 32.9 | 32.8 |
| 12β | 1.68 | 1.69 | 13 | 47.8 | 47.8 |
| 14 | 1.70 | 1.71 | 14 | 49.5 | 49.4 |
| 15α | 1.86 | 1.84 | 15 | 23.5 | 23.5 |
| 15β | 1.52 | 1.50 | 16 | 37.0 | 36.9 |
| 16α | 2.04 | 2.04 | 17 | 83.6 | 83.7 |
| 16β | 2.06 | 2.06 | 18 | 14.2 | 14.1 |
| $CH_3$ | 1.02 | 1.01 | 20 | 140.0 | 141.8 |
| 20 | 6.74 | 6.65 | 21 | 125.3 | 122.9 |
| 21 | 6.85 | 7.0 | 22 | 142.1 | 137.8 |
| 23 | 7.69 | N/A | 23 | 127.0 | 127.6 |
| 24 | 7.64 | 7.70 | 24 | 125.6 | 125.7 |
| 25 | N/A | 7.61 | 25 | 128.9 | 132.6 |
| 26 | 7.64 | 7.44 | 26 | 125.6 | 127.2 |
| 27 | 7.69 | 7.82 | 27 | 128.2 | 128.0 |
| N/A | N/A | N/A | $CF_3$ | 125.4 | 125.7 |

[a] Additional alkyl: 1, $OCH_3$; 2, $CF_3$; 3, $CH_2OH$

**Table 2.** Relative Energies and Key Dihedrals of Predicted Conformers of **1** and **2** Using MM3

| Conformers | C13-C17-C20-C21 | C20-21-22-23 | Relative Energies (kcal/mol) |
|---|---|---|---|
| **1a** | 161 | -169 | 0 |
| **1b** | -96 | 18 | 0.30 |
| **1c** | 89 | 158 | 0.32 |
| **2a** | 87 | -145 | 0 |
| **2b** | 95 | 151 | 0.06 |
| **2c** | 89 | -56 | 0.92 |
| **2d** | 149 | 144 | 1.25 |
| **2e** | -99 | 55 | 1.26 |
| **2f** | 162 | -148 | 1.33 |
| **2g** | -65 | -49 | 1.53 |
| **2h** | -95 | -68 | 1.59 |

**Table 3.** Experimental and Predicted $^{13}C$ Chemical Shifts (ppm) of Predicted Conformers of **1** Using B3LYP/3-21G(X,6-31+G*)//MM3 Calculations

| Carbon | 1a | 1b | 1c | expt |
|---|---|---|---|---|
| C1 | 127.6 | 127.2 | 127.3 | 126.4 |
| C2 | 113.2 | 113.1 | 113.1 | 112.9 |
| C3 | 153.3 | 153.1 | 153.0 | 155.3 |
| C4 | 116.1 | 115.8 | 115.7 | 115.3 |
| C5 | 136.4 | 136.0 | 135.9 | 137.7 |
| C6 | 31.2 | 30.8 | 30.7 | 30.0 |
| C7 | 28.2 | 28.3 | 28.3 | 27.7 |
| C8 | 40.3 | 39.8 | 39.5 | 40.1 |
| C9 | 44.2 | 43.9 | 43.9 | 44.0 |
| C10 | 131.6 | 131.5 | 131.8 | 131.3 |
| C11 | 28.3 | 28.1 | 28.2 | 26.7 |
| C12 | 31.4 | 31.8 | 32.4 | 32.9 |
| C13 | 46.5 | 48.1 | 48.1 | 47.8 |
| C14 | 50.2 | 48.0 | 47.9 | 49.5 |
| C15 | 25.7 | 26.2 | 26.4 | 23.5 |
| C16 | 45.9 | 36.8 | 39.2 | 37.0 |
| C17 | 83.0 | 84.9 | 84.1 | 83.6 |
| C18 | 15.2 | 16.6 | 15.5 | 14.2 |
| C20 | 149.2 | 144.9 | 143.4 | 140.0 |
| C21 | 133.9 | 131.6 | 131.3 | 125.3 |
| C22 | 137.9 | 138.6 | 137.6 | 142.1 |
| C23 | 121.6 | 122.4 | 122.0 | 127.0 |
| C24 | 127.5 | 127.6 | 127.5 | 125.6 |
| C25 | 132.2 | 131.7 | 131.8 | 128.9 |
| C26 | 128.0 | 127.8 | 127.8 | 125.6 |
| C27 | 129.3 | 128.5 | 129.2 | 128.2 |
| C28 | 130.8 | 130.9 | 130.8 | 125.4 |

**Table 4.** Experimental and Predicted $^{13}C$ Chemical Shifts (ppm) of Predicted Conformers of **2** Using B3LYP/3-21G(X,6-31+G*)//MM3 Calculations

| Carbon | 2a | 2b | 2c | 2d | 2e | 2f | 2g | 2h | expt |
|---|---|---|---|---|---|---|---|---|---|
| C1 | 127.4 | 127.6 | 127.3 | 127.5 | 127.5 | 127.4 | 127.6 | 127.6 | 126.4 |
| C2 | 113.1 | 113.1 | 113.0 | 113.1 | 113.1 | 113.1 | 113.1 | 113.0 | 112.9 |
| C3 | 153.1 | 152.9 | 153.0 | 153.0 | 153.0 | 153.1 | 153.1 | 152.9 | 155.2 |
| C4 | 115.8 | 115.5 | 115.8 | 115.8 | 115.7 | 115.9 | 115.8 | 115.7 | 115.3 |
| C5 | 136.2 | 135.8 | 136.1 | 136.1 | 136.1 | 136.3 | 136.2 | 136.2 | 137.5 |
| C6 | 31.1 | 30.8 | 30.7 | 31.1 | 31.1 | 30.9 | 31.1 | 31.1 | 29.9 |
| C7 | 28.3 | 28.4 | 28.1 | 28.3 | 28.3 | 28.2 | 28.4 | 28.3 | 27.7 |
| C8 | 39.9 | 39.7 | 40.1 | 39.9 | 39.5 | 40.1 | 40.3 | 40.0 | 40.0 |
| C9 | 44.3 | 44.4 | 44.2 | 44.3 | 44.3 | 44.3 | 44.5 | 44.2 | 44.0 |
| C10 | 131.6 | 132.1 | 131.6 | 131.6 | 132.0 | 131.5 | 131.7 | 132.1 | 131.3 |
| C11 | 28.3 | 28.5 | 28.5 | 28.2 | 28.3 | 28.2 | 28.4 | 28.4 | 26.6 |
| C12 | 33.0 | 33.8 | 33.1 | 31.9 | 32.2 | 31.9 | 29.4 | 30.5 | 32.8 |
| C13 | 48.1 | 48.6 | 48.0 | 47.7 | 47.2 | 47.2 | 48.6 | 47.6 | 47.8 |
| C14 | 51.5 | 51.1 | 50.9 | 49.6 | 48.3 | 49.7 | 50.8 | 47.6 | 49.4 |
| C15 | 26.0 | 26.5 | 26.0 | 25.8 | 26.4 | 25.6 | 26.8 | 26.1 | 23.5 |
| C16 | 39.6 | 42.4 | 39.2 | 39.6 | 38.9 | 41.6 | 39.7 | 37.6 | 36.9 |
| C17 | 83.6 | 83.9 | 84.6 | 84.0 | 83.6 | 84.0 | 81.9 | 85.5 | 83.7 |
| C18 | 16.3 | 15.0 | 16.1 | 16.3 | 15.2 | 16.3 | 18.3 | 16.9 | 14.1 |
| C20 | 146.2 | 146.7 | 151.2 | 146.3 | 151.1 | 147.5 | 149.6 | 151.2 | 141.8 |
| C21 | 130.7 | 133.3 | 130.1 | 129.9 | 225.7 | 132.5 | 128.7 | 131.7 | 122.9 |
| C22 | 137.2 | 137.5 | 140.8 | 138.6 | 140.4 | 138.7 | 140.7 | 140.6 | 137.8 |
| C23 | 126.8 | 126.4 | 128.1 | 127.3 | 128.4 | 126.6 | 128.7 | 129.0 | 127.6 |
| C24 | 131.9 | 131.9 | 130.7 | 132.1 | 130.9 | 131.9 | 130.9 | 131.0 | 125.7 |
| C25 | 130.6 | 130.6 | 130.9 | 130.9 | 131.0 | 130.7 | 130.9 | 130.8 | 132.6 |
| C26 | 127.8 | 127.6 | 129.5 | 127.7 | 129.5 | 127.7 | 129.3 | 129.0 | 127.2 |
| C27 | 130.5 | 130.1 | 131.4 | 130.1 | 131.2 | 130.0 | 131.0 | 130.5 | 128.0 |
| C28 | 126.8 | 126.5 | 126.0 | 126.3 | 126.2 | 126.6 | 126.3 | 126.1 | 125.7 |

**Table 5.** Summary of the Multiple Independent Variable Regression Analysis[a] of the Calculated $^{13}C$ Shifts of Predicted Conformers of **1** and **2**

| Conformer | Estimate (%) | Standard Error (%) |
|---|---|---|
| **1a** | 13 | 29 |
| **1b** | 72 | 32 |
| **1c** | 15 | 28 |
| **2a** | 0 | 14 |
| **2b** | 0 | 13 |
| **2c** | 33 | 18 |
| **2d** | 0 | 18 |
| **2e** | 65 | 33 |
| **2f** | 0 | 30 |
| **2g** | 0 | 4 |
| **2h** | 2 | 22 |

[a] Constraints: Each conformer is greater than or equal to 0 %. Each of the conformer sets **1a-1c** and **2a-2h** total to 100 %.

**Table 6.** Summary and Comparison of Observed NOE enhancements with Expected NOE Intensities[a] for Predicted Conformers of **1**

| Irradiated | Enhanced | 5a | 5b | 5c | Expt |
|---|---|---|---|---|---|
| H20 | 12α | s | s | w | s |
| H20 | H14 | s | s | s | s |
| H20 | 15α | w | n | w | w |
| H20 | 16α,β | w | n | w | w |
| H21 | 12α | n | n | s | n |
| H21 | H14,12β | n | s | w | w |
| H21 | 15α | n | w | n | w |
| H21 | 16α,β | n | s | n | s |

a. Expectations of strong (s), weak (w), and no (n) NOE enhancements correspond to H-H distances of 0 - 2.99; 3.0 - 4.99; and > 5 Å.

**Table 7.** Summary and Comparison of Observed NOE enhancements with Expected NOE Intensities[a] for Predicted Conformers of **2**

| Irradiated | Enhanced | 6a | 6b | 6c | 6d | 6e | 6f | 6g | 6h | Expt |
|---|---|---|---|---|---|---|---|---|---|---|
| H20 | 12α | w | w | w | s | s | s | w | w | s |
| H20 | H14,12β | s | s | s | s | w | s | n | s | s |
| H20 | 15α | w | w | w | s | n | w | n | n | w |
| H20 | 16α,β | s | s | s | w | n | w | w | n | w |
| H21 | 12α | s | w | w | n | n | n | s | w | n |
| H21 | H14,12β | w | w | w | n | s | n | s | w | w |
| H21 | 15α | n | n | n | n | w | n | w | w | w |
| H21 | 16α,β | n | n | n | w | s | w | s | w | s |

a. Expectations of strong (s), weak (w), and no (n) NOE enhancements correspond to H-H distances of 0 - 2.99; 3.0 - 4.99; and > 5 Å.

**Evaluation of 17α-E-(Trifluoromethylphenyl)vinyl Estradiols as Novel Estrogen Receptor Ligands**

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**Abstract:**

As part of our program to develop novel ligands for the estrogen receptor we synthesized the series of isomeric 17α-(trifluormethyl)phenylvinyl estradiols using our solid-phase organic synthesis methodology. The compounds were evaluated for their relative binding affinity (RBA) using the ERα-LBD and *in vivo* potency using the immature rat uterotrophic growth assay. The ortho-isomer had the highest RBA values, 48-223, and the highest estrogenicity *in vivo*. The other isomers had significantly lower affinities and were weaker agonists in the uterotrophic assay. The results suggest that introduction of substituents at the 17α-position of estradiol are tolerated by the ER-LBD and permit agonist responses in the intact animal, however, the effect is sensitive to the position of groups on the phenyl ring. This study demonstrates that the 17α-position of estradiol is a reasonable site for modification but the position and physicochemical properties of such modifications may significantly affect the affinity and efficacy of the ligand.

**Key words:**

Estrogens

(Trifluoromethyl)phenylvinyl Estradiol

Relative Binding Affinity

Uterotrophic Growth

SAR

## 1. Introduction

Breast cancer is the most common cancer diagnosis among women in the United States (1). Approximately 60% of those patients have tumors that are classified as hormone-responsive, meaning that the tissue contains elevated levels of the estrogen receptor and the tumor cell proliferation is stimulated by estrogens (2). Hormonal therapy has been shown to produce a positive objective response (3-7), however, such interventions are often accompanied by serious undesirable side effects that are tolerated because of the particular risks associated with the primary disease. For the past 10 years, studies with anti-estrogens structurally related to tamoxifen (Figure 1) have demonstrated that some of the side effects can be ameliorated, depending upon the features incorporated with in the structure of the drug. Nonsteroidal anti-estrogens that block the cancer cell proliferation without eliminating the beneficial bone density and cardio-protective effects have been termed Selective Estrogen Receptor Modulators (SERMs)(8-12). Steroidal anti-estrogens, e.g., RU 58668 and ICI 182,780(13-14), generally possess a higher affinity for the estrogen receptor than the nonsteroidal antagonists, however, because they produce an anti-estrogenic response in all tissues, the beneficial effects of estrogens are lost. As a result, efforts continue to develop steroidal agents that may exhibit a SERM-profile.

Our research group undertook the development of new therapeutic entities that, in addition to their anti-neoplastic effects, may also serve as prophylactic agents. Our approach was based on the structure-activity relationships (SARs) generated in our earlier studies(15-19) as well as upon the recently published crystal structures of the liganded estrogen receptor ligand binding domain (ER-LBD) (20-23). Our studies indicated that the 17$\alpha$-(halo- and phenyl-)vinyl estradiols had

higher relative binding affinities (RBAs) than anticipated based upon previous SARs (24-26). In essence, the results suggested that the ER tolerated relatively large substituents, i.e., greater than phenylvinyl(styryl) , at the 17α-position that could be exploited in the development of receptor probes or therapeutic agents. Evaluation of the crystal structure of the liganded ER-LBD suggested that these substituents may interact with the helix-12 region of the receptor. Because this segment of the receptor has been associated with mediating some of the agonist and antagonist effects, we developed the hypothesis that the insertion of substituents into that region by our compounds may elicit novel biological effects. Conformational flexibility of helix-12 precluded a reliable prediction regarding which substituents would produce a particular response, and therefore, our research approach would require versatile synthetic methods as well as biological assays that address both receptor affinity and efficacy.

Our research strategy has addressed these two concerns. We have previously described the use of the Stille coupling reaction (27) to introduce functionalized phenyl groups. This reaction is known to tolerate functional group diversity and it proceeds in high yields and under conditions that are amenable to steroid scaffold manipulation. The ortho-, meta- and para-(trifluoromethyl) phenylvinyl estradiol isomers, as well as the unsubstituted E-phenylvinyl estradiol were prepared and characterized as part of our effort to extend our expertise from solution phase organic synthesis to solid-phase organic synthesis(28). In addition, the trifluoromethyl group, because of its combination of inductive, steric and lipophilic charateristics, has been associated with unusual biological properties (29,30). We chose to evaluate receptor binding affinity of the compounds using the ER-LBD isolated from E. coli cells since this method has been reported to give results comparable to that of the ER isolated from rat uterine cytosol, but requiring less manipulation (31). Compounds would then be

evaluated for efficacy using the rat uterotrophic growth assay (32). In this initial study we have compared the *in vitro* and *in vivo* activity of isomeric trifluoromethylated estrogens to that of the unsubstituted phenylvinyl estradiol and demonstrated the significant effects that substitution and position of substitution have upon the estrogen receptor mediated responses.

## 2. Experimental

### 2.1 *In vitro* competitive binding assay

The compounds were screened for their affinity for the ERα-LBD isolated from BL 21 cells that over-expressed the 33kDa pET-23d ERG vector 3(1). Cells were induced with 0.6 mM isopropyl-β-thiogalactopyranoside for 3h at RT, pelleted by centrifugation, frozen and stored at −75 °C. The cells were thawed, and lysed by sonication (4X20 sec) in four volumes of lysis buffer (50 mM Tris, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 M urea, pH 7.4). Clarified fractions, obtained at 30,000 x g for 30 min were pooled, assayed for receptor binding, diluted to 50 nM in ER and 100 μL aliquots were frozen and stored at −75 °C until ready for use. Then 80 μL of the ERα-LBD- containing extract was incubated with 10 μL of 10 nM 6,7-[H-3]-estradiol (specific activity = 51 Ci/mmole) and 10 μL of either buffer, unlabeled estradiol or test ligand in 100 μL total volume. The final concentrations were 1 nM 6,7-[H-3]-estradiol, 2 nM unlabeled estradiol, (using 200 nM estradiol to define specific binding) and 0.5-5000 nM of the test ligand. In all cases, 10 μL of each incubation solution was removed for assay of the actual initial concentration of [H-3]-estradiol and the remainder was incubated at 2 °C or 25° C for 18 hours. After incubation, 100 μL of dextran coated charcoal suspension (fines removed) was added to adsorb the unbound [H-3]-estradiol, incubated for 10 min, centrifuged, and 100 μL samples were taken from the supernatant fraction for assay of radioactivity. The results were calculated and plotted as % specific binding as a function of log of competitor concentration using the best fit equation for the binding inhibition to define 50% inhibition level. Both the curves for the test ligand and that for unlabeled estradiol were required to have a correlation coefficient of >95% to the equation for competitive binding curve before the data was use to

calculate a relative binding affinity (RBA). The RBA was calculated as 100 times [E]/[C], where [E] was the concentration of unlabeled estradiol needed to reduce the specific binding of [H-3]-estradiol by 50% and [C] was the concentration of test ligand needed to reduce the specific binding by 50%.

2.2 Immature rat uterotrophic growth assay

Test ligands were evaluated using the uterotrophic growth assay (32 ). Groups of immature female rats (at least 5 per group) were injected subcutaneously starting with either peanut oil vehicle (control), or part or all of the range of 0.04, 0.156, 0.625, 2.5, 10, 40, 160 or 640 nmoles of test ligand in 0.1 mL peanut oil (with less than 5% ethanol) and the uterine weights were compared to that of rats receiving estradiol for 3 days. Animals were sacrificed 24h after the last injection, uteri were removed, stripped free of fat and connective tissue, weighed wet, dried in vacuo and weighed to dry weight. Curves of uterine weight (wet and dry) vs. amount of compound injected were compared to assess the potency of the test compound vs. estradiol. A similar threee day uterine growth study was used with vehicle control and an estradiol control that compared the uterine weights of animals injected on three doses of the test ligands (based on the estrogenic activity determined in the previous assay) alone, or the test ligand aong with 10 nmoles estradiol. In this case, anti-estrogenic activity was determined by comparing the uterine weights of the animals injected with estradiol and the test compound compared to estradiol alone and test compound alone. The relative estrogenicity of the test ligands to that of estradiol was assessed by determining the dose at which the compound or estradiol gave a uterine growth response equal to 50% of that of 10 nmoles of estradiol. We also calculated the dose at which the test ligand showed a response of 50% of the maiximum seen for that compound to compare the relative estrogenic potency of the test ligands.

## 3. Results and Discussion

### 3.1 *In vitro* binding

The target compounds were prepared using our solid-phase organic synthesis methodology and are described elsewhere (28). The purified compounds were evaluated for their relative binding affinity for the ERα-LBD expressed in BL-21 cells and the results are shown in Table 1 (RBA for estradiol=100). The values at 2 C reflect kinetic effects while the 25°C values represent equilibrium effects (26). In this assay, the order of RBA values at 2° C was ortho(48)>meta-(38)>para- (6) and the unsubstituted E-phenylvinyl estradiol had an RBA=17. Estradiol had a higher affinity than all the compounds, however, the ortho-and meta-trifluoromethylphenylvinyl estradiols were better competitors than the unsubstituted parent compound. At 25 °C the same order of affinity was observed, ortho- (223)>meta- (75)>para- (8), with the unsubstituted compound having RBA = 18, however, the ortho-isomer is now more potent than estradiol and the meta-isomer is only slightly weaker. Clearly, the very modest step of introducing a trifluoromethyl group onto the phenylvinyl substituent had a significant effect on the ability of the ligand to bind to the ERα-LBD.

### 3.2 *In vivo* assay

These substituent effects were more obvious when the compounds were evaluated for their ability to stimulate uterotrophic growth in immature female rats as shown in Figure 2. All three trifluoromethylated compounds behaved as agonists in that at high doses of the ligand they elicited an effect comparable to that of estradiol. The unsubstituted phenylvinyl estradiol did not produce a measurable estrogenic effect in this assay. When given in combination with estradiol, they did not impede the estrogenic response. However, the doses at which the ligand effects were generated varied over thirty-fold. As expected from the binding data, the ortho-trifluoromethyl

isomer was the most potent with an EC50 value of 0.31 nmoles. The meta-isomer, which had a relatively high RBA value compared to the para-isomer, was the least potent with an EC50 value of 11.1 nmoles vs. 10.6 nmoles. Therefore, in addition to the potency variations associated with the placement of the trifluoromethyl substituent, one also observes that isomerism has modified the relationship between in vitro binding and in vivo potency.

3.3 Discussion

The development of new therapeutic agents for the estrogen receptor involves the interplay of several elements- ligand design, synthesis, biological evaluation, and interpretation of results. Our hypothesis, based on the crystal structure of estrogen ligand – ER-LBD complexes suggested that steroidal compounds with substituents extending from the 17α-position of the steroid skeleton would interact with the receptor in ways that may elicit an anti-estrogenic response. The results from the binding studies clearly indicate that both substitution and the location of the trifluoromethyl group on the phenyl ring play significant roles in the ligand-receptor interaction and may be attributed to the properties of the trifluoromethyl group. Sterically, this substituent is slightly larger than a methyl group (30) and therefore its presence may interact favorably (ortho) or unfavorably (para) depending upon the spatial constraints within the receptor. This lipophilic group may extend into a hydrophobic region adjacent to the ortho-position of the phenyl ring leading to enhanced affinity relative to the unsubstituted compound. In addition, ortho-substitution in phenylvinyl groups produces a torsional rotation of the aromatic ring, such that the trifluoromethyl group is oriented toward a different receptor environment than that experienced for the meta- or para isomers (33).

The *in vivo* results from this small series provided some important findings. First, the substituted phenylvinyl estradiols exhibited estrogen receptor agonism whereas the unsubstituted

parent compound was virtually inactive. Presumably the compounds interact in a manner that permits the receptor to elicit appropriate agonist responses (34,35). Second, it is likely that the in vivo potencies reflect receptor-mediated effects as the observed *in vivo* potencies are qualitatively the same as the *in vitro* binding results. Use of the isomeric compounds with virtually identical Log P values and similar biodistributional properties reduces the differences in rates of metabolism and clearance. Therefore, the observed differences in potency are more likely to reflect of the ability of the compounds to form competent complexes with the receptor.

The results of this study have significant implications for the development of new estrogen-receptor targeted agents, including selective estrogen receptor modulators (SERMs). Nonsteroidal compounds, such as tamoxifen, idoxifene, raloxifene and EM-652, that are very accessible by organic synthesis, produce a variety of complexes with the ERα-LBD, perhaps as a consequence of their structural diversity. The steroidal anti-estrogens, such as RU 58668 and ICI 182,780, have not been extensively evaluated, in part because their syntheses are much more demanding. As a result, the transitions from agonist to antagonist properties within these series are not well characterized. What we have demonstrated in this initial study is a new family of potent estrogen receptor ligands that possesses both synthetic accessibility and structural diversity, and are capable of exerting estrogen receptor mediated effects in vivo. While this study has focused on the ERα-subtype, we are aware of the extensive recent literature concerning the distribution and function of the ERβ-receptor isoformss (36-38). We are extending our work to include the evaluation of these ligands for that receptor as well. More extensive studies to evaluate the effects of substituents on estrogen receptor subtype affinity, selectivity and efficacy are in progress and will be described in subsequent publications.

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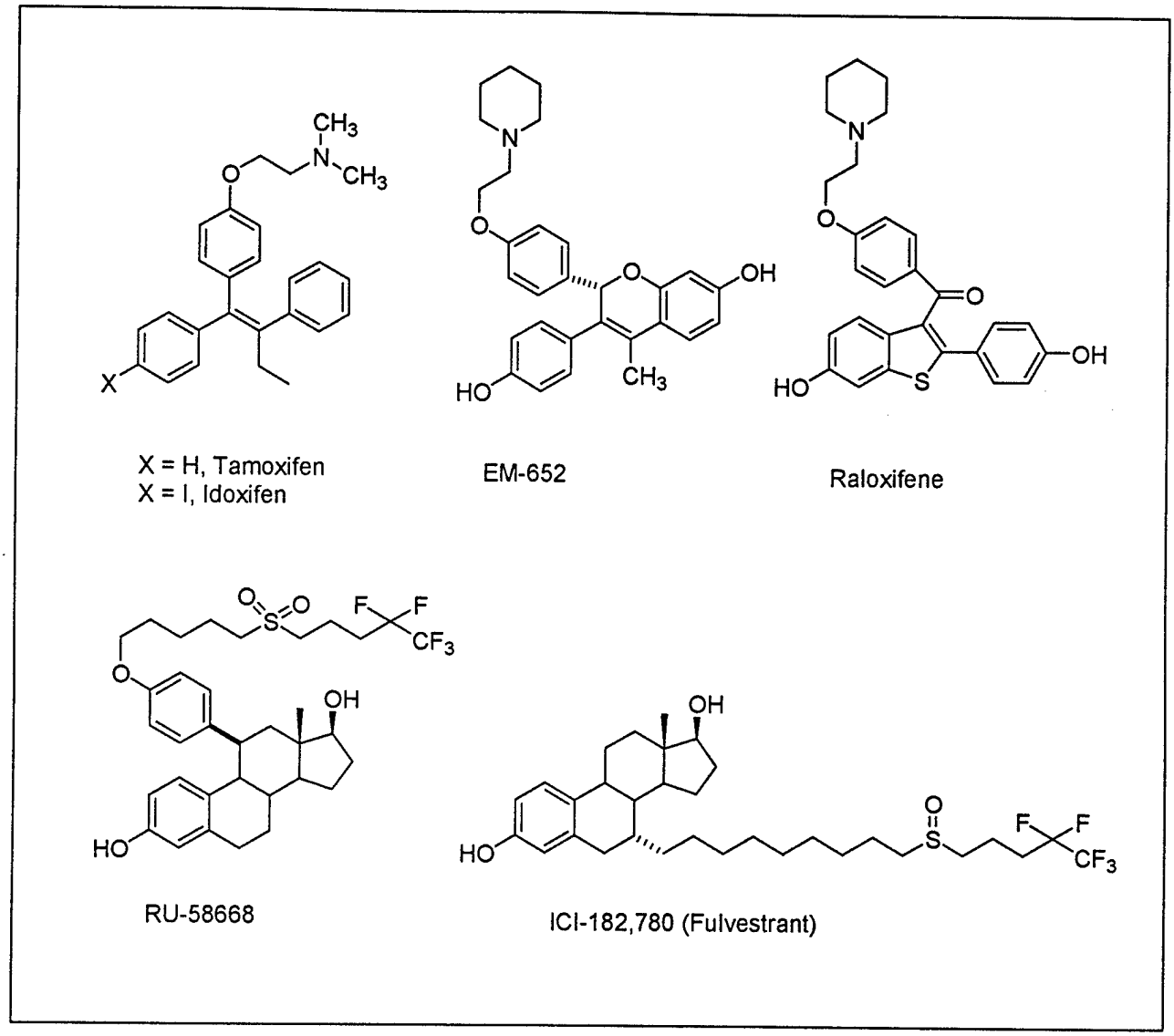

X = H, Tamoxifen
X = I, Idoxifen
EM-652
Raloxifene
RU-58668
ICI-182,780 (Fulvestrant)

Figure 1

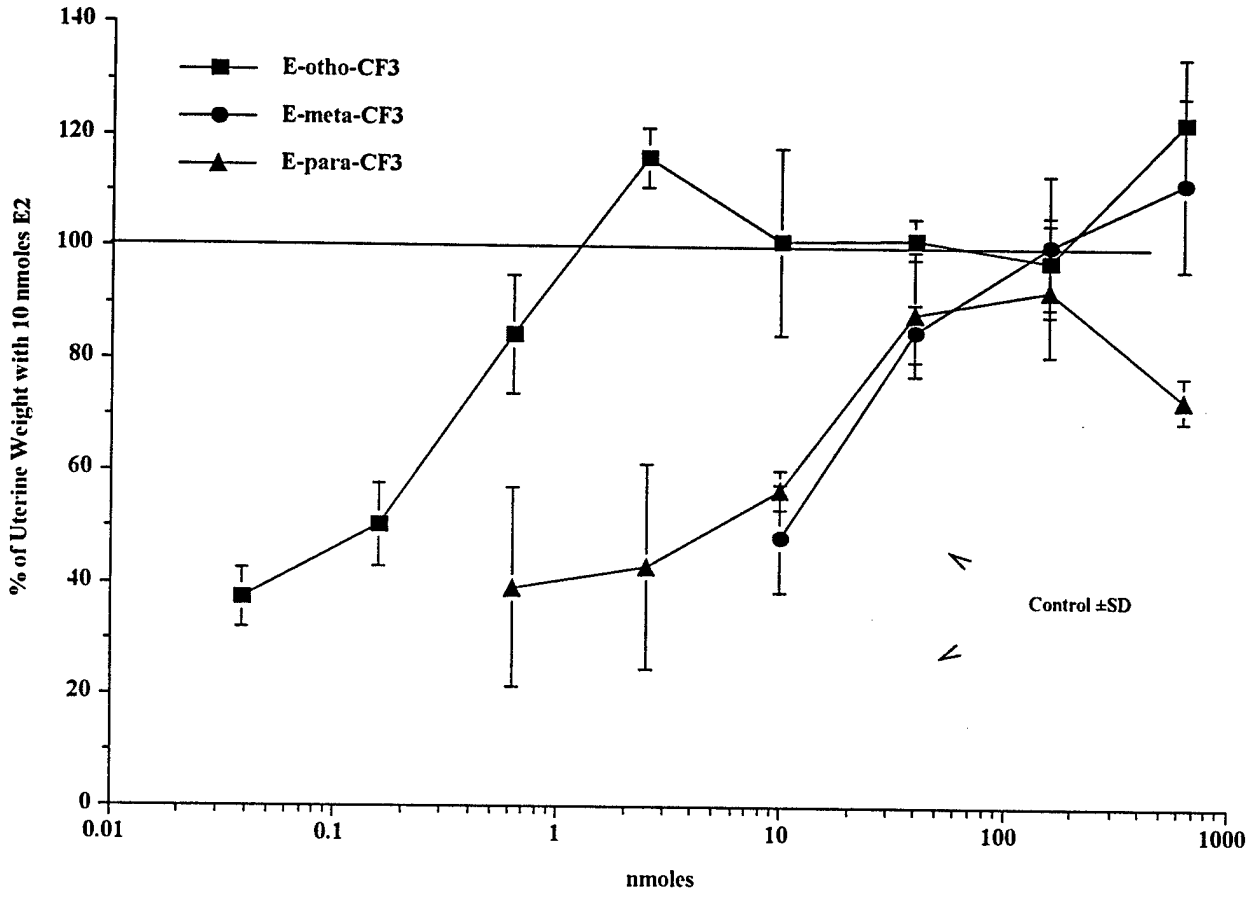
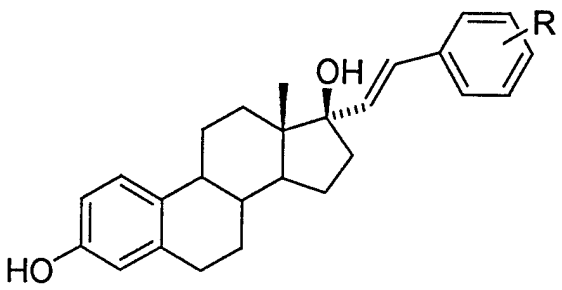

E-otho-CF3
E-meta-CF3
E-para-CF3
Control ±SD
% of Uterine Weight with 10 nmoles E2
nmoles
140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000

Figure 2




| R= | RBA T=2°C | T=25°C 50%$E_2$ | $EC_{50}$(nmoles) 50%max | |
|---|---|---|---|---|
| Estradiol ($E_2$) | 100 | 100 | 0.06 | 0.08 |
| H | 17 | 18 | * | - |
| 2-$CF_3$ | 48 | 223 | 0.31 | 0.42 |
| 3-$CF_3$ | 38 | 75 | 11.1 | 25 |
| 4-$CF_3$ | 6 | 8 | 10.6 | 13 |

RBA (Relative Binding Affinity) = 100 times [E]/[C], where [E] is the concentration of unlabeled estradiol necessary to reduce the specific binding of tritiated estradiol to the ER-LBD by 50% and [C] is likewise the concentration of competitor necessary to reduce the specific binding by 50%. The RBA of estradiol = 100 at each incubation temperature. The ER-LBD was extracted from BL21 cells overexpressing the 33 kDa pET-23d-ERG vector (ref 30).

$EC_{50}$ (nmoles)-50% $E_2$ = dose in nmoles at which the uterine weight in the 4-day assay corresponded to 50% of that shown by 10 nmoles of estradiol $E_2$ .

$EC_{50}$ (nmoles)-50% max- dose in nmoles at which the uterine weight was 50% of the maximum weight shown for this compound over the dose range studied.

* the uterine weights of animals treated with up to 40nmoles of the unsubstituted compound were not statistically greater than control uteri.

## Figure legends

Figure 1. Structures of representative nonsteroidal selective estrogen receptor modulators (SERMs) and steroidal pure anti-estrogens.

Figure 2. Uterotrophic growth assay of the three 17α-E-trifluoromethylphenylvinyl estradiols in immature female rats. The compounds are compared over a 0.04-640 nmole range against a 10 nmole estradiol standard dose. The unsubstituted phenylvinyl estradiol (not shown) did not differ significantly from the control (peanut oil vehicle) range.

**Solid-Phase Synthesis of a Series of 17α-(4-Carboxamidophenyl)vinyl Estradiols and their Evaluation as Estrogen –Receptor Ligands.**

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**Abstract:** A series of 4-carboxamido derivatives of 17α-E-phenylvinyl estradiol was synthesized using a solid-phase organic synthesis (SPOS) approach. The products were obtained in 20-83 % isolated yields and evaluated for their affinity for the estrogen receptor-alpha ligand binding domain (ERα-LBD). The results indicated that although the affinity of the derivatives is less than that of the 4-methoxycarbonyl (methyl ester analog), the binding pocket possesses significant tolerance for larger substituents.

Breast cancer is the most common cancer diagnosis among women with an estimated 200,000 new cases identified per year (1). Of these, over 60% are characterized as hormone-responsive, meaning that they contain elevated levels of estrogen receptors and that tumor proliferation is stimulated by circulating estrogens (2). The relationships between endogenous estrogen production, the binding of estrogens to their nuclear receptors and the expression of disease have been the subject of intense scrutiny for the past 20 years. The cloning of the human estrogen receptor (hER), the determination of its amino acid sequence (3) and the structural homology to the other hormonal nuclear receptors (4) stimulated numerous efforts to develop compounds that could selectively bind to the ER and antagonize or modulate its effects, particularly in neoplastic disease. The recent successes in co-crystallizing ligands with the ER-ligand binding domain (ER-LBD) provided an excellent opportunity to explain how the binding of agonists and antagonists to the ER-LBD could generate the observed biological responses. This understanding could then be used to design agents specifically for the ER (5-8). To date however, such synthetic efforts based on the crystal structures have not been entirely successful. Although we have utilized the crystal structures to guide our efforts, the flexibility of the ER-LBD in the key D-ring binding region of the receptor precluded a focused synthetic strategy. An alternate approach to ER-related drugs involves a combinatorial chemistry to generate a directed library, using the steroidal skeleton as a scaffold on which to append diverse functional groups. Several investigators have used this strategy to prepare and evaluate inhibitors of steroid biosynthesis and metabolism (9-13). In this study we applied the methods we developed for estrogen receptor probes to prepare a new series of steroid derivatives. Evaluation of this series suggests that the receptor tolerates relatively large functional groups at the 17$\alpha$-position and warrants further study.

Two earlier studies provided the basis for the current investigation. As part of our overall program to probe the estrogen receptor and identify potential chemotherapeutic agents we developed a solid phase organic synthesis (SPOS) method for preparing 17$\alpha$-(substituted phenyl)vinyl estradiols (14). Our initial work described the preparation of mono-substituted derivatives 1 while the second publication reported on the extension to a second generation of estrogens 2 (Figure 1). More recently we have described the synthesis and evaluation of a larger series of (4-substituted phenyl) vinyl estradiols in which the 4-methoxycarbonyl derivative 3 exhibited a relative binding affinity (RBA) 18-26% that of estradiol (15). The high affinity of this compound coupled with its *in vivo* activity as a full estrogenic agonist stimulated us to explore the effect of amide substitution on receptor binding.

Our approach to the synthesis of the target 4-carboxamido phenylvinyl estradiols is shown in Scheme I. Our strategy involved the use of the activated ester of resin-bound 17α-E-(4-carboxyphenyl)vinyl estradiol **8** which would then react with the amine of choice. For the preparation of the key functionalized resin we envisioned the use of the trimethylsilyl ethyl ester as the protecting group during the Stille coupling reaction because cleavage of more stable esters, e.g., methyl esters, using sodium hydroxide or sodium methoxide would also effect cleavage from the resin. Cleavage of the trimethylsilyl ethyl ester with tetrabutylammonium fluoride would retain the steroid scaffold on the resin while generating the free carboxylic acid which could then be coupled using standard methods, e.g., DCC, 1-HBT and amine. We chose to evaluate a small set of carboxamides to represent the various possibilities. The N-methyl amide **9a** would be an analog of the methyl ester, N-benzyl **9b** the smallest aralkyl derivative and (S)-N-phenylglycine methyl ester **9c**, the simplest aromatic amino acid derivative.

Preparation of the stannylated resin **4** was achieved in two steps using our published procedure (14). 2-Trimethylsilylethyl 4-iodobenzoate **6** was prepared in 71% yield from trimethylsilylethanol and 4-iodobenzoic acid **5** using DCC-DMAP (17) and then coupled to the resin-bound stannylvinyl estradiol **4** using the Stille procedure in 79% yield (18). The coupling yield was determined by taking an aliquot and cleaving the ester from the resin and isolating the 17α-E-(4-carboxyphenyl)vinyl estradiol **10** in 83% yield. (18). The resin bound (4-carboxyphenyl)vinyl estradiol **8** was obtained using tetrabutylammonium fluoride in THF. This intermediate was then coupled to the appropriate amine and then cleaved from the resin using sodium hydroxide in methanol-dioxane. The products were isolated and purified by column chromatography in 20-70% yields. The products were characterized by H-1 and C-13 NMR and elemental analysis to confirm identity (20-22), and submitted for biological evaluation .

The new compounds were evaluated for their receptor binding affinity at 2°C and 25°C using the hER-LBD isolated from BL21 cells expressing the 33kDa PER-23d ERG vector (16). The results of the binding study are shown in Table 1with the data for estradiol (RBA=100), 17α-E-phenylvinyl estradiol **1** (R=H) the (4-methoxycarbonylphenyl) and (4-carboxyphenyl)vinyl estradiols **3** and **10** included for comparison. Replacement of the methyl ester by the methyl amide causes a substantial decrease in affinity, almost comparable to that of the carboxylic acid. Addition of the phenyl group (N-benzyl) partially restores affinity but binding is still low. Incorporation of the α-methoxycarbonyl group to give the phenylglycine methyl ester derivative yields a product with an RBA virtually identical to the N-benzyl product.

Our preliminary molecular modeling and docking studies of the 4-substituted phenyl vinyl estradiol suggested that the ER-LBD possessed tolerance to small to medium sized substituents at that position (15). Flexibility in the side chains of the involved amino acids would accommodate various functional groups that we put at that position. We hoped that the introduction of appropriate functional groups may provide new interactions that would substantially increase receptor affinity or modify the biological response of the receptor. The preliminary results obtained in this study indicate that the groups chosen for evaluation significantly reduced but did not abolish receptor binding. The fact that the largest substituent had a higher affinity than the smallest suggests that there is reason to believe that appropriate groups would introduce that desired factor. The ease with which the syntheses were conducted and the versatility the solid phase organic synthesis methodology provide a strong basis for undertaking a more concerted effort in this area.

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17. Para-iodo benzoic acid **5** (1.82 g, 7.33 mmol) was dissolved in $CH_2Cl_2$-DMF (3:1). To the solution was added DCC (1.5 g, 7.28 mmol) and the mixture was stirred for 10 min. 2-trimethylsilyl ethanol (0.73 g, 6.18 mmol) was added to the mixture and the mixture was stirred for 2 h at room temperature. On completion of the reaction, the mixture was chromatographed on silica gel to give the product **6** (1.52 g, 4.38 mmol, 71 %) as an oil. $R_f$= 0.93 (hexane- ethyl acetate, 5:1). $^1$H-NMR (300MHz, $CDCl_3$): δ0.00 ppm (s, 9H, Si-($C\underline{H}_3$), 1.05 (t, 2H, J= 8.4Hz, $C\underline{H}_2$), 4.33 (t, 2H, J= 8.4HZ, $OC\underline{H}_2$), 7.65 (d, 2H, J= 8.4Hz, $C_2$-H & $C_6$-H), 7.71 (d, 2H, J=8.7Hz, $C_3$ & $C_5$-H). $^{13}$C-NMR 1.44 (Si-($\underline{C}H_3)_3$), 17.40 ($OCH_2\underline{C}H_2$-Si), 63.53 ($O\underline{C}H_2CH_2$-Si), 100.44 ($C_1$), 130.15($C_4$), 130.95 ($C_3$ & $C_5$), 137.65 ($C_2$ &$C_6$), 166.23 ($\underline{C}$=O).

18. **17α-20E-21-(4-carbotrimethylsilylethoxyphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene -3, 17β-diol 7.** Ethynyl estradiol (3 g, 10.2 mmol) was hydrostannylated in the regular flask at room temperature for 48 h. The swelled resin (2.0 g, 2.47 mmol/g) was treated with carbodiimidazole until the bubbling stopped and the hydrostannylated mixture was transferred to the resin slurry. The reaction mixture was kept under an $N_2$ environment at room temperature for 10 h giving 4.54 g, 2.17 mmol/g of stannylated-vinyl stradiol, **4** , 87.% yield (calculated by dry weight difference). (2-trimethylsilyl)ethyl 4-iodobenzoate 6 was coupled to the hydrostannylated resin **4** by the Stille reaction to afford 17α-20E-21-(4-carbotrimethylsilylethoxybenzoyl)-19-

norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol 7 on the resin (1.72 mmol/g, 79 % yield). FT-IR ($cm^{-1}$) of the compound on the resin, 3630, 3411, 2925, 1720 (C=OOH, sharp), 1655, 1604, 1492, 1440, 1172, 751 $cm^{-1}$. The resins were treated with 1M-tetra-butylammonium fluoride in THF (3 mL). After 5 h, the resins were rinsed with THF and $CH_2Cl_2$ repeatedly and then treated with 5% acetic acid in THF (10 mL). The resins were rinsed with THF and $CH_2Cl_2$ and dried. FT-IR after the deprotection: 3430, 3025, 2927, 1942, 1720 (C=OOH, wide), 1654, 1605, 1492, 1451, 1277, 1178, 1104, 1016, 851, 754, 698, 538 $cm^{-1}$ Subsequently, an aliquot of the resins (0.5 g) was cleaved with 5N NaOH in methanol (2 mL). The combined rinses from the cleavage were condensed under reduced pressure and then partitioned between $CH_2Cl_2$ and water with 1 mL of an aqueous 5 %acetic acid added. Subsequent prep TLC separation afforded 0.30 g (83 % yield) of 17α-20E-21-(4-carboxyphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol **10**.

19. **17α-20E-21-(4-(N-methyl)-benzamido)-19-norpregna-1, 3, 5, (10), 20-tetraene- 3, 17β-diol 9a.** The generated free carboxy terminal of compound **8** above on the swelled resin was treated with DCC for 10 min and then methyl amine was added in the presence of 1-HOBt as a catalyst to generate compound **9a**. $R_f$= 0.30 (hexane-acetone, 2:1), 20 % yield, amorphous. $^1$H-NMR (300 MHz, CDCl3): δ 0.99 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.28 (s, 3H, $C_{29}$-H), 2.7-2.9 (m, 2H, $C_{6\alpha}$-H & $C_{6\beta}$-H), 5.93 (s, 1H, N$\underline{H}$), 6.55 (d, 1H, J=16.2Hz, CH=$C_{21}\underline{H}$), 6.63 (d, 1H, J= 15.9 Hz, $C_{20}\underline{H}$=CH), 6.79 (d, 1H, J= 2.4 Hz, $C_4$-H), 6.82 (dd, 1H, J= 8.4 Hz, $C_2$-H), 7.25 (d, 1H, J= 9.9 Hz, $C_1$-H), 7.46 (d, J= 8.4 Hz, 2H, $C_{23}$-H & $C_{27}$-H), 7.71 (d, 2H, J= 8.1 Hz, $C_{24}$-H & $C_{26}$-H).

20. **17α-20E-21-(4-(N-benzyl)-benzamido)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol 9b.** Compound **8** attached to the resin was treated with carbodiimidazole and allowed to stand until the bubbling stopped. Benzyl amine was added to the slurry. The reaction produced both E-and Z- isomers (compound 32 and 33). $R_f$= 0.46 (chloroform-methanol, 95:5), 70 % yield, mp 155-158 °C, elemental analysis $C_{34}H_{38}O_{3.5}N_1$ (Calc. 79.07 % C, 7.36 % H; Found, 79.52 % C, 7.69 % H). $^1$H-NMR (300 MHz, CDCl3): δ 0.98 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.64 (s, 1H, $C_{17\beta}$-O$\underline{H}$), 2.7-2.9 (m, 2H, $C_{6\alpha}$-H & $C_{6\beta}$-H), 4.65 (d, 2H, J= 5.7 Hz, $C_{29}$-H), 5.93 (s, 1H, $C_3$- OH), 6.51 (t, 1H, J= 5.1 Hz, N$\underline{H}$), 6.54 (d, 1H, J= 16.2 Hz, CH=$C_{21}\underline{H}$), 6.56-6.63 (m, 3H, $C_2$-H, $C_4$-H & $C_{20}$-H), 7.08 (d, 1H, J= 8.1 Hz, $C_1$- H), 7.29-7.36 (m, 5H, $C_{31, 32, 33, 34, 35}$-H), 7.44 (d, 2H, J= 8.1 Hz, $C_{23}$-H & $C_{27}$-H), 7.75 (d, 2H, J= 8.1 Hz, $C_{24}$-H & $C_{26}$-H). $^{13}$C-NMR (75.4 MHz, CDCl3): δ

14.50 ppm ($C_{18}$), 23.71 ($C_{15}$), 26.57 ($C_{11}$), 27.68 ($C_7$), 29.91($C_6$), 32.88 ($C_{12}$), 37.39 ($C_{16}$), 39.78 ($C_8$), 43.91 ($C_9$), 44.48 ($C_{29}$), 47.90 ($C_{13}$), 49.79 ($C_{14}$), 84.26 ($C_{17}$), 112.68 ($C_2$), 115.22 ($C_4$), 126.67 ($C_{33,1}$), 126.80 ($C_{32,34}$), 127.59 ($C_{31,35}$), 127.91 ($C_{25}$), 128.19 ($C_{24,26}$), 129.05 ($C_{23,27}$), 132.46 ($C_{10}$), 132.89 ($C_{21}$), 137.49 ($C_{30}$), 138.28 ($C_{20}$), 138.32 ($C_5$),140.80 ($C_{22}$), 153.58 ($C_3$),167.10 (C=O).

21. **17α-20E-21-(4-(N-carbomethoxybenzyl)-benzamido)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol 9c.** The same method used for the compound **9b** was applied to generate compound **9c**. $R_f$= 0.35 (chloroform-methanol, 95:5), 38 % yield, mp 183-185°C, elemental analysis $C_{36}H_{40}O_{5.5}N_1$ (Calc. 75.26 % C, 6.96 % H; Found, 75.06 % C, 7.26 % H). $^1$H-NMR (300 MHZ, acetone-$d_6$): δ 1.01 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.61 (s, 1H, $C_{17\beta}$-O$\underline{H}$), 2.7-2.9 (m, 2H, $C_{6\alpha}$-H & $C_{6\beta}$-H), 3.71 (s, 3H, $C_{37}$: OC$\underline{H}_3$), 5.75 (d, 1H, J= 7.2 Hz, $C_{29}$-H), 6.52 (d, 1H, J= 3 Hz, $C_4$-H), 6.57 (dd, 1H, J= 8.7 Hz, $C_2$-H), 6.69 (d, 1H, J= 16.2 Hz, CH=$C_{21}\underline{H}$), 6.79 (d, 1H, J= 15.9 Hz, $C_{20}\underline{H}$=CH), 7.06 (d, 1H, J= 8.4 Hz, $C_1$-H), 7.36-7.42 (m, 3H, $C_{32, 33, 34}$-H), 7.51 (dd, 2H, J= 7.5 Hz, $C_{31}$-H & $C_{35}$-H), 7.55 (d, 2H, J= 8.4 Hz, $C_{23}$-H & $C_{27}$-H), 7.93 (d, 2H, J= 8.4 Hz, $C_{24}$-H & $C_{26}$-H), 7.97 (s, 1H, $C_3$-O$\underline{H}$), 8.21 (d, 1H, J= 6.0 Hz, N$\underline{H}$). $^{13}$C-NMR (75.4 MHZ, acetone-$d_6$): δ 14.73 ppm ($C_{18}$), 24.12 ($C_{15}$), 27.25 ($C_{11}$), 28.30 ($C_7$), ($C_6$), 33.51 ($C_{12}$), 37.49 ($C_{16}$), 40.68 ($C_8$), 44.58 ($C_9$), 48.40 ($C_{13}$), 50.08 ($C_{14}$), 52.61($C_{37}$: O$\underline{C}H_3$), 57.97 ($C_{29}$), 84.16 ($C_{17}$), 113.47 ($C_2$), 115.83 ($C_4$), 126.48 ($C_1$), 126.89 ($C_{32, 33, 34}$), 128.66 ($C_{31,35}$), 128.78 ($C_{24,26}$), 129.06($C_{25}$), 129.45 ($C_{23,27}$), 131.91 ($C_{10}$), 133.00 ($C_{21}$), 137.56 ($C_{30}$), 138.31 ($C_{20}$), 139.54 ($C_5$), 141.88 ($C_{22}$), 155.80 ($C_3$), 166.77 ($C_{36}$: $\underline{C}$=O), 171.89 ($C_{28}$: $\underline{C}$=O).

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# Synthesis and Evaluation of (17α, 20E)-21-(4-Substituted-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols as Probes for the Estrogen Receptor-alpha (ERα) Hormone Binding Domain

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Abstract:

As part of our program to develop probes for the hormone binding domain of the estrogen receptor alpha ERα, we prepared a series of 4-(para)-substituted phenyl vinyl estradiol derivatives using a combination of solution and solid phase Pd(0) catalyzed methods. The compounds were evaluated for their binding affinity using the ERα- hormone binding domain (HDB) isolated from transfected cells. The results indicated that although the new compounds were somewhat lower in binding affinity than estradiol, most had higher affinity than the unsubstituted parent phenyl vinyl estradiol. The series was evaluated using molecular modeling and molecular dynamics to determine key interactions between the ligand, especially the para substituent, and the protein. The results suggest that the observed relative binding affinities are directly related to the calculated binding energies, and that amino acids juxtaposed to the para position play a significant but not dominant role in binding. Modification in the properties and/or position of the aryl substituents will be undertaken in subsequent series to further define that role.

**Introduction:**

Breast cancer is the most common cancer diagnosis among women, with an estimated 181,000 new cases per year in the United States (1). Approximately 60% of these newly diagnosed patients have hormone-responsive breast cancer, defined as containing estrogen receptor (ER) and requiring the presence of circulating estrogens for maintenance of tumor growth (2). This relationship has generated considerable interest both for understanding the mechanism of the hormone-receptor interactions and for targeting the ER in therapeutic breast cancer drug development. Recent publications of the crystal structure of the liganded ER-HBD have suggested that the key interaction may involve the N-terminal region (helix-12) of the receptor (3-6). Antagonists apparently cause this helical region of the ER-HBD to occupy a different binding mode compared to that produced by agonists, thereby disrupting the interaction between the receptor and the co-activator proteins that initiate the agonist response (7-10). Because the orientation of the helix-12 of the ER-HBD may be affected differently by various ligands, a variety of approaches can be used to generate compounds that can bind effectively to the receptor protein and subsequently produce the desired pharmacological response. Most strategies have involved modifications of the nonsteroidal antagonists tamoxifen and raloxifene (11-20), however, other groups who have used a heterocyclic moiety to replace the ethylene bridge have also been successful in preparing interesting ER ligands (21-24).

As part of our program to develop new probes for the estrogen receptor, we have focused on the preparation and evaluation of novel steroidal derivatives. Our approach involved the introduction of substituents at the 17α-position of estradiol as a means to enhance receptor binding and/or alter receptor response .Our initial studies described the synthesis and evaluation of several series of 17α-phenylvinyl estradiols. These studies, conducted prior to the publication of the first ER-HBD crystal structures, suggested that there was significant tolerance for large functional groups at this site (25-29). Later reviews of the structure-activity relationships for ER-ligands supported these observations and provided a rationale for the orientation of the 17α-substituent within the ER-HBD (30-32). In order to appreciate these observations we used molecular modeling to have dock our initial 17α-(E)-phenylvinyl estradiol with the ER-HBD and performed energy minimization to identify potential interactions. This model, in which we have oriented the steroid nucleus in the same manner as that found for the estradiol-ER complex, provided two significant points. The 17α-group was accommodated within the outer portion of the domain and

relatively close to the hinge between helices-11 and –12. The substituent was also close to Met-421, a residue that is one of two amino acids that is different from that found in the binding region of the ER-beta (ERβ) isoform (9,33-35). Therefore, we proposed that the introduction of substituents onto the 17α-phenylvinyl group would provide information regarding the interactions between ligands and the estrogen receptor isoforms. However, the model of the interaction between the ligand and the receptor could not predict either the magnitude of the effects of additional substituents on the terminal aromatic ring or the effect on the orientation of the helix-12 and by extension, the biological response.

As part of our ongoing investigation into the ER-HBD and its ligand interactions we have undertaken the preparation of new 17α-substituted phenylvinyl estradiol derivatives in which the substituents would probe the receptor surface. The phenylvinyl group provides six degrees of variation, i.e., E-vs Z- stereochemistry around the C-C double bond as well as 2-,3-,4-substitution on the phenyl ring. In this report we describe the synthesis, receptor binding and computational analysis of a series of 4-substituted 17α-E-phenylvinyl estradiol derivatives. The reasons for this selection included synthetic concerns as well as conformational issues. Our experience with the Stille coupling reaction indicated that our synthetic approach via the vinylstannane and the commercially available (or readily accessible) para-substituted aryl halides would easily generate a series of compounds with a variety of functional groups (36). Ultimately we expected to extend the solution phase chemistry to our solid phase organic synthesis strategy for combinatorial chemistry (37,38). Of equal importance, was the recognition that para-substitution would yield products that would be symmetrical along the aryl axis. This would reduce the number of potential conformational isomers in which the compound could exist and simplify modeling the interactions between the ligand and the receptor. Our preliminary NMR studies with the substituted phenylvinyl estradiols (E- and Z-isomers) indicated that the compounds existed in a conformational equilibrium with a relatively low energy barrier between them (39,40). Therefore, incorporating the conformational mobility of the ligand into the docking interaction with the receptor would be simplified by the use of the para-substitution. As our results suggest, the presence of the substituent and its properties had a significant effect on the binding of the ligand to the ER-HBD.

**Results:**

**Synthesis of estrogenic ligands.**

The target compounds in this series were prepared as part of a larger program to probe ligand-receptor interactions and to develop potential theraputic agents. As a result, we utilized several methods to obtain the compounds. The synthesis of most of the 17α-E-(4-substituted phenyl)vinyl estradiols (5a-5g) was achieved using the solution phase Stille coupling approach developed in our laboratories (Scheme I). The commercially available ethynyl estradiol 1 was acetylated to give the 3-acetyl intermediate 2 (41) which was then hydrostannated with tri-n-butyltin hydride and tri-ethyl borane to give predominantly the E-stannylvinyl estradiol 3. The acetylated intermediate was then coupled with the 4-substituted aryl halides using standard Stille coupling conditions to yield the intermediates 4a-4f. Hydrolysis with sodium methoxide in methanol provided the target 17α-E-(4-substituted phenyl)vinyl estradiols 5a-5f while saponification of 5f provided the carboxy derivative 5g.

[Insert Scheme 1]

Alternatively, as part of our combinatorial chemistry approach, ethynyl estradiol 1 was hydrostannated to give predominately the E-stannylvinyl estradiol 6 which was coupled to a carboxylated polystyrene resin to give the intermediate 7. Stille coupling with the appropriate aryl halide followed by cleavage from the resin gave the target estradiol derivatives 5h-i (Scheme 2).

[Insert Scheme 2]

[Insert Scheme 3]

A third approach utilized the Suzuki coupling reaction (42,43). This involved first performing iododestannylation of 3 to give the iodovinyl estradiol 8 which underwent facile Suzuki coupling with 4-fluorophenyl boronic acid to give, after hydrolysis, the product 5j. The products were purified by column chromatography, recrystallized, and characterized by NMR and MS or elemental analysis. Stereochemistry of the products was established by the coupling constant for the vinylic protons was J= 16-18 Hz, consistent with the previously synthesized E-(trans) derivatives (27).

**Receptor Binding Studies**

The new compounds were evaluated for their ERα-HBD binding affinity at 2° and 25° C using the protein isolated from the transfected BL21 cells. The ligands were compared to both estradiol and the

unsubstituted phenylvinyl estradiol using this assay and the results, shown in Table 1, indicated that all of the compounds retained significant affinity for the estrogen receptor. Although none of the new compounds bound as potently as estradiol, the range of relative binding affinities straddled that of the unsubstituted phenylvinyl estradiol (RBA = 16 at 2 °C and 9 at 25° C). At 2° C, the derivatives with the highest affinity were the 4-acetyl (RBA= 53), 4-methoxy (RBA = 36), 4-hydroxy (RBA = 21) and 4-fluoro (RBA =20) phenylvinyl estradiols. At 25° C, the best ligands were the 4-acetyl (RBA = 60), 4-methoxy (RBA = 32), 4-fluoro (RBA = 28), 4-cyano (RBA = 27), 4-methoxycarbonyl (RBA = 26), and 4- hydroxy (RBA = 25) phenylvinyl estradiols. The only compound with RBA values significantly less than that of phenylvinyl estradiol at either temperature was the polar 4-carboxy derivative 5g (RBA = 1-2).

**Molecular Modeling Studies.**

Molecular modeling of the ligands and the ligand-ERα-HBD complexes was undertaken to interpret the relationship between the structure of the compounds and their receptor binding affinity. Earlier studies with estrogenic ligands (44,45) focused on compounds that were either substituted directly on the A-D rings or were nonsteroidal analogs of estrogens. As such, the results were not directly applicable to our work, even though the approaches were similar.

The results of our molecular modeling/dynamics study are depicted in Figures 1 and 2 and Table 2. The docking experiments indicated two low energy modes, as previously noted (44), however, only the complexes similar to the crystal forms were evaluated in this study. Docking with the unsubstituted phenylvinyl estradiol gave a complex in which the 17α-substituent generated new potential interactions with the sidechains of the ER-HBD. The two edges of the phenyl ring interact with different residues, however, conformational mobility around the phenyl-vinyl axis would allow an ortho- or meta-substituent to select its individual low energy conformation. Para-subsituents, on the other hand, are independent of the rotation of the phenyl group around the double bond and would interact with a common set of residues. As our model indicates, this set consists of several methionine residues, notably Met-342, 348, and 421, plus Phe-425. This is consistent with recent evaluations of ligand-ER-LBD complexes. (46). The other amino acids associated with the ligand-receptor binding have been identified from earlier studies, i.e., Phe-404, Glu-353, and Arg- 394, and interact similar to the other ligands. Our calculations suggest that the

introduction of the phenylvinyl substituent causes the methionines and the phenylalanine sidechains to be displaced by 1-2 A upon binding. The methylthio- groups of the methionines form a cage around the phenyl ring with the para-position now oriented toward the junction of Met-342 and Met-421. The introduction of substituents at this position has relatively little effect on the torsion angle between the phenyl ring and the C-C double bond. The conformation of the substituent is primarily affected by the local environment of the HBD adjacent to the para- position of the phenyl ring. As the Figure 1 demonstrates, small groups, such as the 4-F, CN, methyl, trifluoromethyl, and hydroxy are easily accommodated within the space and establish few interactions. Larger groups, such as the 4- acetyl, methoxycarbonyl, carboxy and methoxy, are required to undergo torsional motion to establish a low energy conformation within the HBD. This equilibration is reflected not only in the final orientation of the substituent, but also in the translational motion of amino acid side chains in the vicinity of the ligand. These movements are ultimately reflected in the calculated binding energies for the complexes.

**Discussion.**

We have prepared a series of 4-substituted phenylvinyl estradiols and evaluated them as probes for the ERα-ligand binding domain. The methods used for the synthesis of the target compounds were chosen to demonstrate the feasibility of each approach and do not represent the optimal conditions. The target compounds 5a-j were obtained in reasonable yields and in high purity by a combination of solution and solid phase palladium-catalyzed reactions, illustrating the versatility and flexibility of this strategy. We screened the new compounds with the ERα-HBD and certain 4-substituted derivatives displayed high relative binding affinity (RBA) for the ERα-HBD with values in the range of 25-60%, exceeding that of the unsubstituted parent. Docking the new ligands in the ER-HBD using molecular modeling suggests that the substituted phenylvinyl group is easily accommodated by the outer portion of the ligand binding pocket.

**Structure-activity relationships in the 4-substituted phenylvinyl series.** . Previous studies in our laboratories have shown that the estrogen receptor tolerated the introduction of 17α-X-vinyl substituents. Although the highest affinity was observed for the halovinyl estradiols, phenyl- and phenylthio/selenovinyl estradiols also were good ligands. Studies of the topography of the ER-

LBD with halovinyl estrogens are limited by the small number of substitutions that are available while the phenylthio/selenovinyl estrogens are restricted both by the availability of substituted reagents for electrophilic destannylation and the rotation around the S/Se-vinyl bond. Introduction of substituents on the phenylvinyl group via the versatile Pd-catalyzed Stille or Suzuke reactions made the phenylvinyl estrogens an excellent method for investigating the ligand-receptor interactions. The substituents that we have introduced at the 4-position included electron-withdrawing as well as electron-donating, hydrophilic as well as hydrophobic, small as well as large groups. Virtually all of the new compounds are comparable in their ER affinity relative to the unsubstituted parent compound, except for the 4-trifluoromethyl and 4-carboxy-compounds. The rest had affinities that were roughly 20-60% that of estradiol compared to 9-16% for the unsubstituted compound 5a. This increase in binding was essentially independent of the properties of the substituent, for example, the 4-hydroxy compound was virtually identical to the 4-cyano and 4-methoxycarbonyl derivatives (RBA = 25 vs 27 vs 26), and 4- fluoro similar to 4-methyl (RBA = 22 vs 18). The highest affinity was observed for the 4-acetyl derivative (RBA = 60), although its properties are similar to the methyl ester (RBA = 26) or the methyl ether (RBA = 32). The lack of a clear relationship between structure and affinity suggested to us that in the process of binding, both the ligand and receptor were undergoing structural adjustments to reach an energy minimum. An analysis of this type of interaction would best be achieved using molecular modeling and docking studies.

**Investigation of the interactions of the 17α-4-substituted phenylvinyl estradiols with the ER-HBD. Molecular modeling studies.** We used molecular modeling and molecular dynamics to investigate the interactions between the phenylvinyl substituent of our ligands and the amino acid sidechains of the ER-HBD. We chose the co-ordinates of the estradiol-ERα-HBD complex because of the steroidal nature of our compounds and because preliminary biological data indicated that the compounds behaved as agonists in the immature rat uterotrophic assay. Therefore, the orientation of the critical helix-12, associated with co-activator binding, was probably in the agonist orientation. Using the modeling program with the Insight II package (47), we docked the 4-unsubstituted phenylvinyl estradiol into the estradiol binding site, overlaying the

aromatic rings. Employing molecular mechanics and energy minimization routines, approximately 20 low energy conformers were obtained for each complex. In each case, the17α-substituent was oriented toward the external surface of the receptor. The translations of the internal amino acids associated with the A-B-C-ring interactions were relatively small, consistent with the crystal structures obtained with the other estrogen receptor agonists and with the steroidal and nonsteroidal androgens at the androgen receptor (48,49). This effect has also been observed with the vitamin D analog-vitamin D receptor-HBD crystal structures where the internal structure remains relatively rigid while the sidechain of the analog tends to undergo the conformational deformations (50-52). In our model, the phenylvinyl substituent occupied a region bounded by three methionines (Met-342,343,421), a phenylalanine (Phe-425) as well as two leucines (Leu-346,410) and a valine (Val-418). While relatively lipophilic in character, these residues also can interact through the electron pairs of the thio ether and/or through the π-cloud of the phenyl ring. Therefore, substituents present at the para-position of the phenylvinyl group can experience multiple effects. Analysis of individual amino acids indicated that the highest contribution to binding energy derived from Phe-404 and Leu-387 via direct interactions with the α- and β-faces of the A-ring. The second highest contribution arose from Leu-346 that interacts directly with both the steroidal C-ring and the phenyl vinyl group. Met-421 is closest to the 17α-phenyl group while Met-342 and Met 343-juxtapose the para- and vinyl groups, respectively. If one includes the consideration that steric factors could influence translational or torsional responses on these sidechains, then the interpretation of the individual effects gets increasingly complex. As shown in Figure 2, the overlap of the ligands (deleting the ER-HBD) shows that the substituents occupy a reasonably small volume in which electronegativity is not as critical as substituent conformation. As a result, the methionines tolerate a polar substituent (fluoro-, carbonyl-) adjacent to the phenyl ring as long as the next group is lipophilic (-methoxy, -methyl).

There are several conclusions that can be drawn from this study. First, ERα-HBD can accommodate the presence of a significant variety of substituents at the para-position of the phenylvinyl estradiols. This finding had not been previously observed and leads to the possibility functional groups can be introduced that may impart higher receptor affinity, selectivity or altered

efficacy. Second, molecular modeling and molecular dynamics have provided a method for not only evaluating the interactions between ligands and the receptor hormone binding domain, but, at least within a homologous (para-substituted) series, possibly predicting the affinity of putative ligands. Lastly, we have demonstrated the feasibility of Pd(0) coupling methods to prepare the diverse members of such a series a may be required to identify a potential clinical candidate. Subsequent publications in this project will describe those efforts to extend these methods to other series in the phenylvinyl estradiol family.

## EXPERIMENTAL

**General Methods**. All reagents and solvents were purchased from Aldrich or Fisher Scientific. THF and toluene were distilled from sodium/benzophenone. Reactions were monitored by TLC, performed on 0.2 mm silica gel plastic backed sheets containing F-254 indicator. Visualization on TLC was achieved using UV light, iodine vapor and/or phosphomolybdic acid reagent. Column chromatography was performed with 32-63μm silica gel packing. Melting points were determined using an Electrotherm capillary melting point apparatus and are uncorrected. NMR spectra chemical shifts are reported in parts per million downfield from TMS and referenced either to TMS internal standard for deuterochloroform or deuteroacetone solvent peak. Coupling constants are reported in hertz. All compounds gave satisfactory elemental analyses, ± 0.4%,(Atlantic Microchemical Laboratories, Inc. Norcross, GA) unless otherwise stated.

**Solution Phase Synthesis**

**17α-ethynyl estradiol 3-acetate 2** . 17α-ethynyl estradiol 1 (2.5 g) was dissolved in a mixture of pyridine (15 mL) and acetic anhydride (2.5 mL) and stirred at room temperature for 4 h. The solution was poured into ice water and the mixture was allowed to stand at the room temperature for 1 h. The resulting precipitate was collected by filtration and washed with water. The solution was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure (41). Recrystallization from acetone-hexane afforded a 98 % yield. $R_f$= 0.24 (hexane-ethyl acetate, 5:1), mp 145-147 °C. $^1$H-NMR (300 MHz, $CDCl_3$): δ 0.88 ppm (s,

3H, $C_{18}$ $C\underline{H}_3$), 1.2-2.4 (steroid envelope), 2.28 (s, 3H, $C\underline{H}_3C$=O), 1.3 (s, 1H, -C≡ $C_{21}$-$\underline{H}$), 2.7-2.9 (m, 2H, $C_{6\alpha}$-$\underline{H}$ & $C_{6\beta}$-$\underline{H}$), 3.48 (s, 1H, $C_{17\beta}$-O$\underline{H}$), 6.78 (d, 1H, J=2.5Hz, $C_4$-H), 6.84 (dd, 1H, J= 2.6, 8.5 HZ, $C_2$-H), 7.29 (d, 1H, J= 8.3 HZ, $C_1$-H). $^{13}$C-NMR (75.4 MHz, $CDCl_3$): δ 12.60 ppm (C18), 21.10 ($\underline{C}H_3C$=O), 22.75 (C15), 26.17 (C11), 26.98 (C7), 29.49 (C6), 32.66 (C12), 38.90 (C16), 38.98 (C8), 43.67 (C9), 47.01 (C13), 49.43 (C14), 74.04 (C21), 79.77 (C17), 87.45 (C20), 118.54 (C2), 121.45 (C4), 126.38 (C1), 137.85 (C10), 138.18 (C5), 148.36 (C3), 169.86 ($CH_3\underline{C}$=O).

**17α-E-(tri-n-butylstannyl)-vinyl estradiol 3-acetate 3**. To a solution of 3-acetoxy- 17α-ethynyl-estradiol 2(1.5 g, 4.4 mmol) in THF (5 mL) were added 1.7 mL (6.3 mmol) of tri-n-butyltin hydride and 3 mL (26 mmol) of triethylborane. The reaction mixture was stirred magnetically for 5 h at 60°C (46) and then purified by column chromatography on silica gel using hexane-ethyl acetate (5:1) as the eluent. The reaction afforded 0.5 g (0.79 mmol) of 3-acetoxy-17α-Z-(tri-n-butylstannyl)-vinyl estradiol and 1.89 g (3 mmol) of 3-acetoxy-17α-E-(tri-n-butylstannyl)-vinyl estradiol in a combined yield of 86 %. $R_f$ (Z-isomer) = 0.58 (hexane-ethyl acetate, 5:1), $R_f$ (E-isomer) = 0.43 (hexane-ethyl acetate, 5:1), amorphous. $^1$H-NMR (300MHZ, $CDCl_3$): δ 0.88 ppm (s, 3H, $C_{18}$ $C\underline{H}_3$), 1.2-2.4 (steroid envelope), 2.28 (s, 3H, $C\underline{H}_3C$=O), 2.7-2.9 (m, 2H, $C_{6\alpha}$-$\underline{H}$ & $C_{6\beta}$-$\underline{H}$), 6.06 (d, 1H, J= 19.4 Hz, CH=$C_{21}\underline{H}$), 6.21 (d, 1H, J=19.4 Hz, $C_{20}\underline{H}$=CH), 6.79 (d, 1H, J=2.4 Hz, $C_4$-H), 6.84 (dd, 1H, J= 2.6, 8.4 HZ, $C_2$-H), 7.28 (d, 1H, J= 8.8 HZ, $C_1$-H). $^{13}$C-NMR (75.4 MHz, $CDCl_3$): δ 9.64 ppm ($C_{22}$, 4C), 13.78 (C24, 4C), 14.18 ($C_{18}$), 21.13 ($\underline{C}H_3C$=O), 23.43 ($C_{15}$), 26.15 ($C_{11}$), 27.28 ($C_{25}$, 4C), 27.37 ($C_7$), 29.20 ($C_{23}$, 4C), 29.59 ($C_6$), 32.35 ($C_{12}$), 35.87 ($C_{16}$), 39.05 ($C_8$), 44.06 ($C_9$), 46.61 ($C_{13}$), 49.06 ($C_{14}$), 85.47 ($C_{17}$), 118.54 ($C_2$), 121.48 ($C_4$), 124.68 ($C_{21}$), 126.39 ($C_1$), 138.05 ($C_{10}$), 138.27 ($C_5$), 148.38 ($C_{20}$), 152.40 ($C_3$), 169.89 ($CH_3\underline{C}$=O).

**Method I. General procedures for the synthesis of 4a-4g. Stille coupling.** To a solution 3-acetoxy-17α-E-(tri-n-butylstannyl)-vinyl estradiol 3 (0.5 mmol) in dry toluene (5 mL) were added the aryl halide (Br/I) (0.6-0.7 mmol) and a catalytic amount (5.0 mg) of tetrakis(triphenylphosphine)palladium (0) and 3 crystals of 3,5-di-tert-butyl –4-hydroxytoluene. The reaction was stirred for 10 h at 90-100°C under a nitrogen atmosphere. The reaction was cooled to ambient temperature and filtered to remove catalyst. The filtrate was concentrated by rotary evaporation, dissolved in ethyl acetate (50 mL) and washed sequentially with

saturated ammonium chloride, saturated potassium fluoride, and brine. The organic layer was dried over mangnesium sulfate (anhyd.), filtered and evaporated to dryness. The residue was purified by column chromatography on silica gel using hexanes-ethyl acetate or chloroform-methanol as the eluent.

**General procedure for deacetylation. Synthesis of 5a-5g, 5j.** The purified 3-acetoxy-17α-(4-substituted phenyl)-vinyl estradiols were dissolved in methanol containing 0.4 mL of 10N sodium hydroxide (or sodium methoxide for 5f). The solution was stirred for 2h, then acidified with dilute acetic acid (4%) and partitioned between ethyl acetate and water. The organic phase was washed with 10% sodium bicarbonate, dried over magnesium sulfate, filtered and evaporated to dryness. The crude product was purified by column chromatography on silica gel using hexanes-ethyl acetate. The final compounds were crystallized from hexanes-acetone(ethyl acetate) to provide analytical samples for the binding studies.

**Method II. General procedure for solid phase synthesis 5h,5i.** The stannylated resin was placed in the reaction vessel and swelled with dichloromethane. The solvent was removed a evacuation and the resin was treated with dry toluene (10 mL). To the slurry were added the appropriate aryl halide (Br/I), 2 –3 crystals of 3,5-di-tert-butyl-4-hydroxytoluene, and a small amount (5 mg) of the Pd(0) catalyst. The reaction was heated at 80-90°C overnight under nitrogen. The reaction was agitated to maintain dispersal of the materials. After cooling to ambient temperature, the resin was washed three times each with dichloromethane, methanol, tetrahydrofuran, and warm dimethyl formamide, dried in vacuo, and characterized by FTIR. The resin was swelled in dichloromethane (10 mL) containing 3 mL 5N sodium hydroxide in methanol and stirred for 1 h. The cleavage step was repeated three times. The solutions were combined, acidified with dilute acetic acid, and partitioned between ethyl acetate and water. The organic phase was washed with 10% sodium bicarbonate, brine, dried over magnesium sulfate, filtered and evaporated to dryness. The crude product was purified by column chromatography on silica gel using hexanes-ethyl acetate as the eluent. The final product was crystallized from hexanes-acetone/ethyl acetate to obtain analytical samples for the binding studies.

**17α-20E-21-phenyl-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol 3-acetate (4a).** 25 % yield, $R_f$ = 0.23 (hexane-ethyl acetate, 5:1). $^1$H-NMR (300 MHZ, $CDCl_3$): δ 0.88 ppm (s, 3H, $C_{18}$-C<u>H</u>$_3$), 1.2-2.4 (m, steroid envelope), 2.28 (s, 3H, C<u>H</u>$_3$C=O-), 2.7-2.9 (m, 2H, $C_{6\alpha}$-<u>H</u> & $C_{6\beta}$-<u>H</u>), 6.48 (d, 1H, J=16.1 Hz, CH=$C_{21}$<u>H</u>), 6.60 (d, 1H, J= 16.1 Hz, $C_{20}$H=CH), 6.80 (d, 1H, J=2.3 Hz, $C_4$-H), 6.83 (dd, 1H, J= 2.4, 8.4

HZ, $C_2$-H), 7.26 (d, 2H, J= 7.4 HZ, $C_1$ & $C_{25}$-H), 7.34 (t, 2H, J= 7.8 Hz, $C_{24}$ & $C_{26}$-H), 7.44 (d, 2H, J= 7.1Hz, $C_{23}$ & $C_{27}$-H). $^{13}$C-NMR (75.4 MHZ, $CDCl_3$): δ 14.10 ppm ($C_{18}$), 21.08 ($\underline{C}H_3C$=O), 23.35 ($C_{15}$), 26.09 ($C_{11}$), 27.17 ($C_7$), 29.50($C_6$), 32.43 ($C_{12}$), 36.85 ($C_{16}$), 39.08 ($C_8$), 43.77 ($C_9$), 47.36 ($C_{13}$), 49.34 ($C_{14}$), 84.03 ($C_{17}$), 118.49 ($C_2$), 121.41 ($C_4$), 126.31 ($C_{25}$), 126.40 ($C_{24}$, $C_{26}$), 127.32 ($C_1$), 127.50 ($C_{21}$), 128.55 ($C_{23}$, $C_{27}$), 134.82 ($C_{10}$), 137.10 ($C_{20}$), 137.94 ($C_5$), 138.14 ($C_{22}$), 148.33 ($C_3$), 169.80 ($CH_3\underline{C}$=O).

**17α-20E-21-phenyl-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol (5a).** The hydrolysis of the 3-actate group afforded the product in a 92 % yield. $R_f$ = 0.18 (hexane-ethyl acetate, 4:1, mp 176-177 °C, $R_f$ = 0.17 (hexane-acetone, 4:1), elemental analysis $C_{26}H_{30}O_2$.0.5$CH_3CO_2C_2H_5$ . $^1$H-NMR (300MHZ, acetone-$d_6$): δ 1.01 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.7-2.9 (m, 2H, $C_{6\alpha}$-$\underline{H}$ & $C_{6\beta}$-$\underline{H}$), 3.77 (s, 1H, $C_{17\beta}$-O$\underline{H}$), 6.52 (d, 1H, J= 2.6 Hz, C4-H), 6.58 (dd, 1H, J= 2.7, 8.4 HZ, $C_2$-H), 6.63 (s, 2H, $C_{20}\underline{H}$=$C_{21}\underline{H}$), 7.07 (d, 1H, J= 8.3 HZ, $C_1$-H), 7.20 (t, 1H, J= 7.2 Hz, $C_{25}$-H), 7.31 (t, 2H, J= 7.7 Hz, $C_{24}$, $C_{26}$-H), 7.46 (d, 2H, J=7.1 Hz, C23, C27-H). $^{13}$C-NMR (75.4 MHz, acetone-$d_6$): δ 14.73 ppm ($C_{18}$), 24.09 ($C_{15}$), 27.28 ($C_{11}$), 28.31 ($C_7$), 33.46 ($C_{12}$), 37.41 ($C_{16}$), 40.71 ($C_8$), 44.62 ($C_9$), 48.29 ($C_{13}$), 50.06 ($C_{14}$), 84.10 ($C_{17}$), 113.52 ($C_2$), 115.89 ($C_4$), 126.98 ($C_{25}$), 127.13 ($C_{24}$& $C_{26}$), 127.38 ($C_1$), 127.70 ($C_{21}$), 129.31 ($C_{23}$ & $C_{27}$), 132.06 ($C_{10}$), 137.24 ($C_{20}$), 138.39 ($C_5$), 138.71($C_{22}$), 155.87 ($C_3$).

**17α-20E-21-(4-hydroxyphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol 3-acetate (4b).** To a solution of 3-acetoxy-17α-E-(tri-n-butylstannyl)-vinyl estradiol (0.35 g, 0.56 mmol) in toluene (5 mL) were added 4-iodophenol (0.15 g, 0.68 mmol), 3 crystals of 3,5-di-t-butyl-4-hydroxytoluene and a catalytic amount (15 mg) of $Pd(PPh_3)_4$. to afford 50 mg of the product. 21 % yield, amorphous.

**17α-20E-21-(4-hydroxyphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol (5b).** Evaporation followed by silica gel column chromatography with 2 % methanol in chloroform afforded the amorphous product in an 89 % yield (0.04 g). elemental analysis $C_{26}H_{30}O_3$-0.5 $CH_3CO_2C_2H_5$. $^1$H-NMR (300 MHZ, acetone-$d_6$): δ 0.86 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.7-2.9 (m, 2H, $C_{6\alpha}$-$\underline{H}$ & $C_{6\beta}$-$\underline{H}$), 3.67 (s, 1H, $C_{17\beta}$-O$\underline{H}$), 6.28 (d, 1H, J= 16 Hz, CH=$C_{21}\underline{H}$), 6.39 (d, 1H, J= 2.7 Hz, $C_4$-H), 6.40 (d, 1H, J=16.1 Hz, $C_{20}\underline{H}$=CH), 6.44 (dd, 1H, J= 2.6, 8.4 HZ, $C_2$-H), 6.66 (d, 2H, J= 8.7 Hz, $C_{24}$, $C_{26}$-H), 6.94 (d, 1H, J= 8.3 HZ, $C_1$-H), 7.17 (d, 1H, J= 8.6 Hz, $C_{23}$-H & $C_{27}$-H), 7.96 (s, 1H, C3-O$\underline{H}$), 8.37 (s, 1H, $C_{25}$-O$\underline{H}$). $^{13}$C-NMR (75.4 MHZ, acetone-$d_6$): δ 14.72 ppm ($C_{18}$), 24.05 ($C_{15}$), 27.29 ($C_{11}$), 28.32 ($C_7$), 33.40

($C_{12}$), 37.26 ($C_{16}$), 40.71 ($C_8$), 44.66($C_9$), 48.16 ($C_{13}$), 49.94 ($C_{14}$), 84.02 ($C_{17}$), 113.43 ($C_2$), 115.80 ($C_4$), 116.07 ($C_{26}$), 116.15 ($C_{24}$), 126.99 ($C_{21}$), 127.21 ($C_1$), 128.33 ($C_{23}$ & $C_{27}$), 130.27 ($C_{22}$), 132.08 ($C_{10}$), 134.02 ($C_{20}$), 138.40 ($C_5$), 155.78 ($C_3$), 157.45 ($C_{25}$).

**17α-20E-21-(4-cyanophenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol 3-acetate (4c).** The purification by silica gel column chromatography using a hexane-ethyl acetate gradient (5/1→3/1) afforded the product in a 50 % yield. $R_f$ = 0.21 (hexane-ethyl acetate, 3:1). $^1$H-NMR (CDCl3, 300MHz): δ0.98 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.28 (s, 3H, C$\underline{H}_3$C=O), 2.7-2.9 (m, 2H, $C_{6\alpha}$-$\underline{H}$ & $C_{6\beta}$-$\underline{H}$), 6.63 (t, 2H, J= 16.6 Hz, $C_{20}\underline{H}$=$C_{21}\underline{H}$), 6.79 (d, 1H, J= 2.3 Hz, $C_4$-H), 6.83 (dd, 1H, J= 2.5, 8.4 HZ, $C_2$-H), 7.24 (d, 1H, J= 8.3HZ, $C_1$-H), 7.49 (d, 2H, J= 8.3 Hz, $C_{23}$-H & $C_{27}$-H), 7.60 (d, 2H, J= 8.3Hz, $C_{24}$-H & $C_{26}$-H). $^{13}$C-NMR (75.4 MHZ, acetone-$d_6$): δ 14.05 ppm ($C_{18}$), 20.99 ($\underline{C}H_3C$=O), 23.31 ($C_{15}$), 25.98 ($C_{11}$), 27.10 ($C_7$), 29.37 ($C_6$), 32.53 ($C_{12}$), 37.12 ($C_{16}$), 38.99 ($C_8$), 43.69 ($C_9$), 47.54 ($C_{13}$), 49.59 ($C_{14}$), 84.03 ($C_{17}$), 110.22 ($C_{25}$), 118.47 ($C_2$), 118.90 ($\underline{C}$≡N), 121.38 ($C_4$), 125.73 ($C_1$), 126.19 ($C_{21}$), 126.81 ($C_{23}$, $C_{27}$), 132.27 ($C_{24}$, $C_{26}$), 137.62 ($C_{10}$), 137.98 ($C_5$), 138.98 ($C_{20}$), 141.68 ($C_{22}$), 148.30 ($C_3$), 169.74 ($CH_3\underline{C}$=O).

**17α-20E-21-(4-cyanophenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol (5c).** The product was purified by silica gel column chromatography with a hexane-ethyl acetate (4:1) eluent. Recrystallization (hexane-acetone) afforded the pure product 12 (0.11 g, 0.26 mmol) in an 80 % yield, $R_f$ = 0.08 (hexane-ethyl acetate, 4:1), mp139-140°C, elemental analysis $C_{27}H_{29}O_2N_1.0.5CH_3CO_2C_2H_5$ ; $^1$H-NMR (300 MHz, acetone-$d_6$): δ 1.01 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.7-2.9 (m, 2H, $C_{6\alpha}$-$\underline{H}$ & $C_{6\beta}$-$\underline{H}$), 3.92 (s, 1H, $C_{17\beta}$-O$\underline{H}$), 6.52 (s, 1H, $C_4$-H), 6.58 (dd, 1H, J=2.7, 8.7 Hz, $C_2$-H), 6.73 (d, 1H, J= 16 Hz, CH=$C_{21}\underline{H}$), 6.90 (d, 1H, J=16 Hz, $C_{20}\underline{H}$=CH), 7.07 (d, 1H, J= 8.3 Hz, $C_1$-H), 7.67 (d, 2H, J= 8.9 Hz, $C_{23}$-H & $C_{27}$-H), 7.71 (d, 2H, J= 8.7 Hz, $C_{24}$-H & $C_{26}$-H), 7.97 (s, 1H, $C_3$-O$\underline{H}$). $^{13}$C-NMR (75.4 MHZ, acetone-$d_6$,): δ 14.72 ppm ($C_{18}$), 24.13 ($C_{15}$), 27.26 ($C_{11}$), 28.30 ($C_7$), ($C_6$), 33.57($C_{12}$), 37.65 ($C_{16}$), 40.69 ($C_8$), 44.55 ($C_9$), 48.54 ($C_{13}$), 50.18 ($C_{14}$), 84.29 ($C_{17}$), 110.76 ($C_{25}$), 113.53 ($C_2$), 115.90 ($C_4$), 119.51 ($\underline{C}$≡N), 125.90 ($C_{21}$), 126.97 ($C_1$), 127.88 ($C_{23}$, $C_{27}$), 131.95 ($C_{10}$), 133.18 ($C_{24}$, $C_{26}$), 138.36 ($C_5$), 141.73 ($C_{20}$), 143.39 ($C_{22}$), 155.89 ($C_3$).

**17α-20E-21-(4-methylphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol 3 acetate (4d).** The product was purified by silica gel column chromatography using a hexane-ethyl acetate (4:1) eluent. 59 %

yield, $R_f$ = 0.26 (hexane-ethyl acetate, 4:1), amorphous. $^1$H-NMR (300 MHz, $CDCl_3$): δ 0.90 ppm (s, 3H, $C_{18}$-C<u>H</u>$_3$), 1.2-2.4 (m, steroid envelope), 2.19 (s, 3H, $C_{28}$-C<u>H</u>$_3$), 2.28 (s, 3H, C<u>H</u>$_3$C=O), 2.7-2.9 (m, 2H, $C_{6\alpha}$-<u>H</u> & $C_{6\beta}$-<u>H</u>), 6.34 (d, 1H, J=16.1 Hz, CH=$C_{21}$<u>H</u>), 6.46 (d, 1H, J= 16 Hz, $C_{20}$<u>H</u>=CH), 6.71 (s, 1H, $C_4$-H), 6.74 (dd, 1H, J= 2.6, 8.3 Hz, $C_2$-H), 7.06 (d, 1H, J= 7.8 HZ, $C_{24}$-H & $C_{26}$-H), 7.16 (d, 1H, J= 8.5 Hz, $C_1$-H), 7.25 (d, 2H, J= 8.2 Hz, $C_{23}$-H & $C_{27}$-H).

**17α-20E-21-(4-methylphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol (5d)**

Recrystallization (hexane-acetone) step afforded the pure product 15 (0.09 g). 60 % yield, $R_f$ = 0.19 (hexane-ethyl acetate, 4:1), mp169-170°C, elemental analysis $C_{27}H_{32}O_2$ .0.5$CH_3CO_2C_2H_5$. $^1$H-NMR (300 MHZ, acetone-$d_6$): δ 1.00 ppm (s, 3H, $C_{18}$-C<u>H</u>$_3$), 1.2-2.4 (m, steroid envelope), 2.30 (s, 3H, $C_{28}$-C<u>H</u>$_3$), 2.7-2.9 (m, 2H, $C_{6\alpha}$-<u>H</u> & $C_{6\beta}$-<u>H</u>), 3.72 (s, 1H, $C_{17\beta}$-OH), 6.52-6.63 (m, 4H, $C_2$-H, $C_4$-H, $C_{20}$<u>H</u>=CH & CH=$C_{21}$<u>H</u>), 7.07 (d, 1H, J= 8.8 Hz, $C_1$-H), 7.13 (d, 1H, J= 7.8 HZ, $C_{24}$-H & $C_{26}$-H), 7.35 (d, 2H, J= 8.1 Hz, $C_{23}$-H & $C_{27}$-H), 7.95 (s, 1H, $C_3$-O<u>H</u>). $^{13}$C-NMR (75.4 MHz, acetone-$d_6$): δ14.73 ppm ($C_{18}$), 21.06 ($C_{28}$), 24.09 ($C_{15}$), 27.29 ($C_{11}$), 28.32 ($C_7$), ($C_6$), 33.45 ($C_{12}$), 37.35 ($C_{16}$), 40.73 ($C_8$), 44.65 ($C_9$), 48.26 ($C_{13}$), 50.04 ($C_{14}$), 84.07 ($C_{17}$), 113.53 ($C_2$), 115.91 ($C_4$), 126.98 ($C_1$), 127.08 ($C_{24}$, $C_{26}$), 127.30 ($C_{20}$), 129.94 ($C_{23}$, $C_{27}$), 132.06 ($C_{10}$), 135.94 ($C_{22}$), 136.15 ($C_{21}$), 137.27 ($C_{25}$), 138.40 ($C_5$), 155.90 ($C_3$).

**17α-20E-21-(4-acetylphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol 3-acetate (4e).** The product was purified by silica gel column chromatography using a hexane-ethyl acetate gradient (8/1→1/1). 89 % yield, $R_f$ =0.18(hexane-ethyl acetate, 3:1). $^1$H-NMR (300 MHZ, $CDCl_3$): δ 1.02 ppm (s, 3H, $C_{18}$-C<u>H</u>$_3$), 1.2-2.4 (m, steroid envelope), 2.28 (s, 3H, $C_3$: C<u>H</u>$_3$-C=O), 2.56 (s, 3H, $C_{29}$: C=OC<u>H</u>$_3$), 2.7-2.9 (m, 2H, $C_{6\alpha}$-<u>H</u> & $C_{6\beta}$-<u>H</u>), 6.60 (d, 1H, J= 16.1 Hz, CH=$C_{21}$<u>H</u>), 6.67 (d, 1H, J= 16.1 Hz, $C_{20}$<u>H</u>=CH), 6.79 (d, 1H, J= 2.3 Hz, $C_4$-H), 6.82 (dd, 1H, J= 2.6, 8.4 Hz, $C_2$-H), 7.24 (d, 1H, J= 8.4 Hz, $C_1$-H), 7.50 (d, 2H, J= 8.4 HZ, $C_{23}$-H & $C_{27}$-H), 7.92 (d, 2H, J= 8.4 Hz, $C_{24}$-H & $C_{26}$-H).

**17α-20E-21-(4-acetylphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol (5e).** The product was purified by recrystallization (hexane-ethyl acetate) to afford 0.27 g (0.65 mmol). 83 % yield, mp149-150°C, $R_f$ = 0.07 (hexane-ethyl acetate, 4:1), elemental analysis $C_{28}H_{32}O_3$ .0.5$CH_3CO_2C_2H_5$. $^1$H-NMR(300 MHZ, acetone- $d_6$): δ 1.02 ppm (s, 3H, $C_{18}$-C<u>H</u>$_3$), 1.2-2.4 (m, steroid envelope), 2.56 (s, 3H, C=OC<u>H</u>$_3$), 2.7-2.9 (m, 2H, $C_{6\alpha}$-<u>H</u> & $C_{6\beta}$-<u>H</u>), 3.88 (s, 1H, $C_{17\beta}$-O<u>H</u>), 6.52 (d, 1H, J= 2.6 Hz, $C_4$-H), 6.58 (dd, 1H, J= 2.7, 8.3 Hz,

$C_2$-H), 6.72 (d, 1H, J= 16 Hz, CH=$C_{21}$H), 6.85 (d, 1H, J= 16 Hz, $C_{20}$H=CH), 7.07 (d, 1H, J= 8.3 Hz, $C_1$-H), 7.60 (d, 2H, J= 8.5 HZ, $C_{23}$-H & $C_{27}$-H), 7.94 (d, 2H, J= 8.5 Hz, $C_{24}$-H & $C_{26}$-H). $^{13}$C-NMR (75.4 MHz, acetone-$d_6$): δ 14.75 ($C_{18}$), 24.15 ($C_{15}$), 26.57 (C=O$\underline{C}$H$_3$), 27.29 ($C_{11}$), 28.33 ($C_7$), 33.57 ($C_{12}$), 37.51 ($C_{16}$), 40.72 ($C_8$), 44.61 ($C_9$), 48.49 ($C_{13}$), 50.20 ($C_{14}$), 84.27 ($C_{17}$), 113.52 ($C_2$), 115.88 ($C_4$), 126.52 ($C_1$), 126.98 ($C_{21}$), 127.19 ($C_{24}$, $C_{26}$), 129.46 ($C_{23}$, $C_{27}$), 132.0 ($C_{10}$), 136.61 ($C_{25}$), 138.39 ($C_5$), 140.49 ($C_{20}$), 143.28 ($C_{22}$), 165.46 ($C_3$), 197.20 ($\underline{C}$=OCH$_3$).

**17α-20E-21-(4-methoxycarbonylphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol 3-acetate (4f).** The product was purified by silica gel column chromatography using a hexane-ethyl acetate gradient (4/1→3/1) in 65 % yield, $R_f$ = 0.28 (hexane-ethyl acetate, 3:1), amorphous. $^1$H-NMR (300 MHz, CDCl3): δ 0.99 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.28 (s, 3H, C$\underline{H}_3$C=O), 2.7-2.9 (m, 2H, $C_{6\alpha}$-$\underline{H}$ & $C_{6\beta}$-$\underline{H}$), 3.91 (s, 3H, C=OOC$\underline{H}_3$), 6.59 (d, 1H, J= 16.1 Hz, CH=$C_{21}$H), 6.66 (d, 1H, J= 16.1 Hz, $C_{20}$H=CH), 6.79 (s, 1H, J= 2.4 Hz, $C_4$-H), 6.82 (dd, 1H, J= 2.6, 8.4 Hz, $C_2$-H), 7.24 (d, 1H, J= 8.7Hz, $C_1$-H), 7.48 (d, 2H, J= 8.4 Hz, $C_{23}$-H & $C_{27}$-H), 8.0 (d, 2H, J= 8.5 Hz, $C_{24}$-H & $C_{26}$-H). $^{13}$C-NMR (75.4MHz, CDCl3): δ 14.14 ppm ($C_{18}$), 21.10 ($\underline{C}$H$_3$C=O), 23.41 ($C_{15}$), 26.10 ($C_{11}$), 27.20 ($C_7$), 29.51 ($C_6$), 32.58 ($C_{12}$), 37.14 ($C_{16}$), 39.12 ($C_8$), 43.80 ($C_9$), 47.56 ($C_{13}$), 49.58 ($C_{14}$), 52.03 (C=OO$\underline{C}$H$_3$), 84.16 ($C_{17}$), 118.55 ($C_2$), 121.46 ($C_4$), 126.30 ($C_{24}$, $C_{25}$, $C_{26}$), 126.61 ($C_1$), 128.75 ($C_{21}$), 129.93 ($C_{23}$, $C_{27}$), 137.67 ($C_{10}$), 137.84 ($C_{20}$), 138.14 ($C_5$), 141.67 ($C_{22}$), 148.38 ($C_3$), 166.88 ($\underline{C}$=OOCH$_3$) 169.83 (CH$_3\underline{C}$=O).

**17α-20E-21-(4-methoxycarbonylyphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol(5f).** The product was purified by silica gel column chromatography using a hexane-acetone system (3:1). Recrystallization using a hexane-ethyl acetate afforded the pure product in a 25 % yield. $R_f$ = 0.19 (hexane-acetone, 3:1). mp 144-145°C, elemental analysis $C_{28}H_{32}O_4$. $^1$H-NMR (300 MHz, acetone-$d_6$): δ 1.01 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.28 (s, 3H, C$\underline{H}_3$C=O), 2.7-2.9 (m, 2H, $C_{6\alpha}$-$\underline{H}$ & $C_{6\beta}$-$\underline{H}$), 3.87 (s, 3H, C=OOC$\underline{H}_3$), 6.53 (s, 1H, $C_4$-H), 6.58 (dd, 1H, J= 2.2, 8.4 Hz, $C_2$-H), 6.72 (d, 1H, J= 16 Hz, CH=$C_{21}$H), 6.85 (d, 1H, J= 15.9 Hz, $C_{20}$H=CH), 7.07 (d, 1H, J= 8.6 HZ, $C_1$-H), 7.60 (d, 2H, J= 8.3 Hz, $C_{23}$-H & $C_{27}$-H), 7.93 (s, 1H, C3-O$\underline{H}$), 7.95 (d, 2H, J= 8.3 Hz, $C_{24}$-H & $C_{26}$-H). $^{13}$C-NMR (75.4 MHz, acetone-$d_6$): δ 14.75 ppm ($C_{18}$), 24.15 ($C_{15}$), 27.29 ($C_{11}$), 28.32 ($C_7$), 33.57 ($C_{12}$), 37.59 ($C_{16}$), 40.73 ($C_8$), 44.60 ($C_9$), 48.49 ($C_{13}$), 50.20 ($C_{14}$), 52.17 (C=OO$\underline{C}$H$_3$), 84.28 ($C_{17}$), 113.55 ($C_2$), 115.92 ($C_4$), 126.48

($C_{25}$), 126.99 ($C_1$), 127.16 ($C_{24}$, $C_{26}$), 129.33 ($C_{21}$), 130.51 ($C_{23}$, $C_{27}$), 132.03 ($C_{10}$), 138.40 ($C_5$), 140.50 ($C_{20}$), 143.41 ($C_{22}$), 155.80 ($C_3$), 167.01 ($\underline{C}$=$OOCH_3$).

**17α-20E-21-(4-carboxyphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3,17β-diol (5g).** Compound 5g was prepared by the same method as compound 5f. 91 % yield, mp 157-158°C, $R_f$ = 0.24 ($CHCl_3$-$CH_3OH$, 95:5); elemental analysis $C_{27}H_{32}O_4$. $^1$H-NMR (300 MHz, acetone-d6): δ 1.02 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.7-2.9 (m, 2H, $C_{6\alpha}$-$\underline{H}$ & $C_{6\beta}$-$\underline{H}$), 6.54 (d, 1H, J= 2.5 Hz, $C_4$-H), 6.59 (dd, 1H, J= 2.6, 8.5 HZ, $C_2$-H), 6.73 (d, 1H, J= 16 Hz, CH=$C_{21}\underline{H}$), 6.84 (d, 1H, J= 16.1HZ, $C_{20}\underline{H}$=CH), 7.06 (d, 1H, J= 8.8 Hz, $C_1$-H), 7.59 (d, 2H, J= 8.3 Hz, $C_{23}$-H & $C_{27}$-H), 8.0 (d, 2H, J= 8.3 Hz, $C_{24}$-H & $C_{26}$-H). $^{13}$C-NMR (75.4 MHz, acetone-$d_6$): δ 14.75 ppm ($C_{18}$), 24.11 ($C_{15}$), 27.23 ($C_{11}$), 28.26 ($C_7$), $C_6$, 33.50 ($C_{12}$), 37.50 ($C_{16}$), 40.65 ($C_8$), 44.52 ($C_9$), 48.45 ($C_{13}$), 50.17 ($C_{14}$), 84.31 ($C_{17}$), 113.51 ($C_2$), 115.89 ($C_4$), 126.57 ($C_{21}$), 126.94 ($C_1$), 127.08 ($C_{24}$, $C_{26}$), 129.59 ($C_{25}$), 130.79 ($C_{23}$, $C_{27}$), 132.01 ($C_{10}$), 138.36 ($C_5$), 140.22 ($C_{20}$), 143.26 ($C_{22}$), 155.78 ($C_3$), 167.58 ($\underline{C}$=OOH).

**Preparation of the resin bound 17α-tri-n-butylstannylvinyl estradiol 7.**

The 17α-ethynyl estradiol 1 (3 g, 10 mmol) was dissolved in THF in a flask and treated with triethylborane (2 mL, 17 mmol) and tributyltin hydride (3 g, 11 mmol). The mixture was stirred at 40°C for 10 h. The reaction mixture was evaporated to dryness, dissolved in $CH_2Cl_2$, and then transferred to the pre-swollen carboxy resin (5 g) in $CH_2Cl_2$ in the presence of DCC. A catalytic amount of DMAP was added to the mixture and the reaction was allowed to stand for 24 h. The total loading yield for the mixture of E-and Z-isomers was 50 % (0.59 mmol/g) comprised of 47 % (0.56 mmol/g) E-isomer and 3 % Z-isomer (0.03 mmol/g).

**17α-20E-21-(4-trifluoromethylphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol (5h).** The product was cleaved and the resulting mixture was purified by silica gel column chromatography using chloroform to afford 0.12 g of the E-isomer product and 1mg of Z-isomer product. 49 % yield, $R_f$ = 0.15 (hexane-ethyl acetate, 4:1), mp 215-217°C, elemental analysis $C_{27}H_{29}O_2F_3$. $^1$H-NMR (300MHz, acetone-$d_6$): δ 1.02 ppm(s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.7-2.9 (m, 2H, $C_{6\alpha}$-H & $C_{6\beta}$-H), 3.90 (s, 1H, $C_{17\beta}$-O$\underline{H}$), 6.53 (d, 1H, J= 2.6 Hz, $C_4$-H), 6.58 (dd, 1H, J= 2.6, 8.4 Hz, $C_2$-H), 6.73 (d, 1H, J= 16 Hz, CH=$C_{21}\underline{H}$), 6.85 (d, 1H, J= 16 Hz, $C_{20}\underline{H}$=CH), 7.07 (d, 1H, J= 8.3 Hz, $C_1$-H), 7.64 (d, 2H, J= 8.7 Hz, $C_{23}$-

H & $C_{27}$-H), 7.70 (d, 2H, J= 8.6Hz, $C_{24}$-H & $C_{26}$-H), 8.0 (s, $C_3$-OH). $^{13}$C-NMR (75.4 MHz, acetone-$d_6$): δ 14.73 ppm ($C_{18}$), 24.13 ($C_{15}$), 27.26 ($C_{11}$), 28.31 ($C_7$), ($C_6$), 33.54 ($C_{12}$), 37.58 ($C_{16}$), 40.69 ($C_8$), 44.58 ($C_9$), 48.46 ($C_{13}$), 50.16 ($C_{14}$), 84.23 ($C_{17}$), 113.53 ($C_2$), 115.90 ($C_4$), 125.44 (q, J= 270.6 Hz, $\underline{C}F_3$), 125.97($C_{21}$), 126.21 (q, J= 3.5 Hz, $C_{26}$), 126.22 (q, J= 3.5 Hz, $C_{24}$), 126.98 ($C_1$), 127.62 ($C_{23}$, $C_{27}$), 128.85 (q, J= 32 Hz, $C_{25}$), 131.98 ($C_{10}$), 138.38 ($C_5$), 140.64 ($C_{20}$), 142.75 ($C_{22}$), 155.88 ($C_3$).

**17α-20E-21-(4-methoxyphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3, 17β-diol (5i).** 36 % yield, $R_f$ = 0.23 ($CHCl_3$-$CH_3OH$, 99:1). Elemental analysis $C_{27}H_{32}O_3$-0.5 $CH_3CO_2C_2H_5$. $^1$H-NMR (300 MHz, acetone-$d_6$): δ 0.99 ppm (s, 3H, $C_{18}$-C$\underline{H}_3$), 1.2-2.4 (m, steroid envelope), 2.7-2.9 (m, 2H, $C_{6\alpha}$-H & $C_{6\beta}$-H), 3.68 (s, 1H, $C_{17\beta}$-OH), 3.78 (s, 3H, OC$\underline{H}_3$), 6.46 (d, 1H, J= 16.1 Hz, CH=$C_{21}\underline{H}$), 6.51-6.59 (m, 3H, $C_2$-H, $C_4$-H, & $C_{20}$-H), 6.88 (d, 2H, J= 8.8 Hz, $C_{24}$-H & $C_{26}$-H); 7.07 (d, 1H, J= 8.3 Hz, $C_1$-H). 7.39 (d, 2H, J= 8.8 Hz, $C_{23}$-H & $C_{27}$-H), 7.95 (s, 1H, $C_3$-O$\underline{H}$). $^{13}$C-NMR (75.4 MHz, acetone-$d_6$): δ 14.74 ppm ($C_{18}$), 24.07 ($C_{15}$), 27.30 ($C_{11}$), 28.33 ($C_7$), ($C_6$), 33.43 ($C_{12}$), 37.32 ($C_{16}$), 40.73 ($C_8$), 44.67 ($C_9$), 48.21 ($C_{13}$), 49.98 ($C_{14}$), 55.49 (O$\underline{C}H_3$), 84.05 ($C_{17}$), 113.54 ($C_2$), 114.73 ($C_{24}$, $C_{26}$), 115.91 ($C_4$), 126.95 ($C_1$), 126.98 ($C_{21}$), 128.26 ($C_{23}$, $C_{27}$), 131.35 ($C_{22}$), 132.07 ($C_{10}$), 134.87 ($C_{20}$), 138.40 ($C_5$), 155.91 ($C_3$), 159.89 ($C_{25}$).

**17α,20E-21-iodo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol 3-acetate(8).** To a solution of 3 (2.36 g, 3.75 mmol) in chloroform: methylene chloride (1:1, 30 mL) was added a slurry of N-iodosuccinimide (1.0 g, 4.4 mmol) in the same solvent solution. The reaction was stirred, under aluminum foil, at 0 °C for 24 hours. The reaction was followed by TLC for the conversion of 3 ($R_f$ = 0.4, hexane: ethyl acetate 5:1) to 8 ($R_f$ = 0.2 same solvent system). The reaction mixture was washed with saturated sodium bicarbonate/water (50 mL). Aqueous and organic layers were separated. Aqueous layer was extracted with chloroform (50 mL x 2). Organic layers were combined washed with brine (50 mL x 2) and water (50 mL x 2), dried over magnesium sulfate and concentrated. The yellow oil was separated on a silica gel column (60 g), covered with aluminum foil, using chloroform: methanol (98: 2) as the eluting solvent to give 8 as a pure white powder (1.62 g, 93 %): $R_f$ = 0.2 (hexane: ethyl acetate 5:1); $^1$H NMR in $CDCl_3$ δ 0.96 (s, 3H, 18-$CH_3$), 1.2-2.9 (m, 15H, steroid nucleus), 6.32 (d, 1H, $J_{21\text{-}20}$ = 14.34 Hz, 21-H), 6.78 (d, 1H, $J_{4\text{-}2}$ = 2.46 Hz, 4-H) 6.84 (dd, 1H, $J_{2\text{-}4}$ = 2.58 Hz, $J_{2\text{-}1}$ = 8.04 Hz, 2-H), 6.88 (d, 1H, $J_{20\text{-}21}$ = 14.22 Hz, 20-H), 7.29 (d, 1H and $CDCl_3$ peak, $J_{1\text{-}2}$ = 8.28 Hz, 1-H); $^{13}$C NMR in $CDCl_3$ δ 14.16 (C-18), 21.17 (-OCO$\underline{C}H_3$), 22.67 (C-

15), 26.08 (C-11), 27.18 (C-7), 29.51 (C-6), 32.47 (C-12), 36.65 (C-16), 39.07 (C-8), 43.77 (C-9), 47.07 (C-13), 49.35 (C-14), 74.72 (C-21), 87.10 (C-17), 118.62 (C-2), 121.52 (C-4), 126.40 (C-1), 137.80 (C-10), 138.15 (C-5), 150.46 (C-3), 148.43 (C-20), 169.92 (-O<u>C</u>O$CH_3$)

**Method III. Suzuki coupling. Synthesis of 17α,20E-21-(4-fluorophenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol 3-acetate (4j).** To a solution of 8 (1.34 g, 2.9 mmol) in THF (10 mL) was added Tris(dibenzylideneacetone)dipalladium (0.25 g, 0.27 mmol), sodium bicarbonate (1.28 g, 12.08 mmols, 4 equiv, in 5 mL water) and 4-fluorobenzeneboronic acid (0.86 g, 6.1 mmol). The reaction mixture was protected from light and stirred at room temperature for 12 hours. The mixture was extracted with ethyl acetate (4 x 100 mL), washed with brine (200 mL) and water (5 x 100 mL), dried over magnesium sulfate, filtered and concentrated to yield a yellow powder. The residue was chromatographed on a silica gel column (50 g) using 98:2 chloroform:methanol as the eluting solvent to give 4j (0.46 g, 37%): $R_f$ = 0.2 (hexane: ethyl acetate 4:1); $^1$H NMR in $CDCl_3$ δ 0.97 (s, 3H, 18-$CH_3$), 1.2-2.9 (m, b, 15H, steroid nucleus), 6.37 (d, 1H, $J_{20\text{-}21}$ = 15.99 Hz, 20-H), 6.54 (d, 1H, $J_{21\text{-}20}$ = 16.11 Hz, 21-H), 6.78 (d, 1H, $J_{4\text{-}2}$ = 2.3 Hz, 4-H), 6.83 (dd, 1H, $J_{2\text{-}4}$ = 2.5 Hz, $J_{2\text{-}1}$ = 8.4 Hz, 2-H), 6.9 (~t, 2H, $J_{24\text{-}F}$ and $J_{26\text{-}F}$ = 8.4 Hz, $J_{24\text{-}23}$ and $J_{26\text{-}27}$ = 6.7 Hz, $J_{24\text{-}27}$ and $J_{26\text{-}23}$ = 2 Hz, 24-H and 26-H), 7.24 (d, 1H and $CDCl_3$ peak, $J_{1\text{-}2}$ = 8.3 Hz, 1-H), 7.37 (m, 2H, 25-H and 27-H)

**(17α,20E)-21-(4-fluorophenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (5j).** Our standard deprotection method of 4j (0.34 g, 0.79 mmol) yielded 5j (0.31 g, 100 %). Recrystallization in hexane:acetone 3:1 produced a fine white powder.(0.31 g, 97%): $R_f$ = 0.17 (hexane: ethyl acetate 4:1); mp 189-191 °C; elemental analysis $C_{26}H_{29}FO_2$-0.5 $CH_3CO_2C_2H_5$. $^1$H NMR in acetone $d_6$ δ 1.0 (s, 3H, 18-$CH_3$), 1.2-2.9 (m, 15H, steroid nucleus), 6.39 (d, 1H, $J_{21\text{-}20}$ = 16.08 Hz, 21-H), 6.55 (d, 1H, $J_{20\text{-}21}$ = 16.02 , 20-H), 6.56 ( d, 1H, $J_{4\text{-}2}$ = 2.79 Hz, 4-H), 6.61 (dd, 1H, $J_{2\text{-}1}$ = 8.4 Hz, $J_{2\text{-}4}$ = 2.82 Hz, 2-H), 7.02 (ddd, 2H, 24-H and 26-H), 7.12 (d, 1H, $J_{1\text{-}2}$ = 8.31 Hz, 1-H), 7.38 (dd, 2H, 23-H and 27-H); $^{13}$C NMR in acetone $d_6$ δ 15.07 (C-18) 24.42 (C-15), 27.60 (C-11), 28.64 (C-7), ~29 under acetone peak (C-6), 33.79 (C-12), 37.78 (C-16), 41.02 (C-8), 44.92 (C-9), 48.61 (C-13), 50.34 (C-14), 84.44 (C-17), 113.86 (C-2), 116.24 (C-4), 116.30 (d, $J_{CCF}$ = 21 Hz, C-24 and C-26), 126.50 (C-21), 127.30 (C-1), 129.15 (d, $J_{CCCF}$ = 7.9 Hz, C-23 and C-27), 132.31 (C-10), 135.50 (C-22), 137.52 (C-20), 138.71 (C-5), 156.18 (C-3), 163.06 (d, $J_{C\text{-}F}$ = 243 Hz, C-25).

**Molecular modeling and dynamics.**

We initially evaluated the conformations of our ligands 5a-j using the Builder module from Insight II. Potentials for each atom were assigned automatically or manually, when necessary. Low energy conformations were generated using the molecular mechanics method (Discover program, 100 steps, 0.001 final convergence) and compared to solution conformations determined by NMR (39). The ER-HBD used in our study was obtained from the Protein Data Bank (PDB ID 1QKU, wild type ERα-HBD co-crystallized with estradiol). Monomer C from the homodimer B/C was selected for the docking and molecular dynamics studies. All water molecules were deleted except for the one positioned near ARG 394 and GLU 353 that is present in all crystal structures. The monomer C contains all the amino acid residues between ASN 304 and HIS 550. All manipulations were performed using the Builder module in Insight II. The complex of ER-LBD monomer and estradiol bound within the binding cavity was minimized using the molecular mechanics method (Discover_3 module, CVFF force field, conjugate gradient minimization 10,000 steps, 0.001 final convergence).

Docking of the ligands with the ERα-HBD was performed using the Docking module in InsightII (47). The ligand was superimposed on the estradiol molecule (A-ring over A-ring) and the estradiol was then deleted. During the docking procedure both the ligand and the protein residues within the ligand binding cavity (amino acids within 15 angstroms of the ligand as well as all amino acids in helix-12, loops 11-12, 1-3, 6-7) were allowed to flex. In addition, the phenylvinyl side chain of the ligand was rotated with 30° increments in order to more fully explore the potential binding modes of the conformational choices of the ligand. After each docking procedure, structures within 10 kcal/mol of the lowest energy structure and RMS distance of more than 0.125 A were selected and used in simulated annealing studies. In this procedure, the structures were subjected to short molecular dynamics runs (100 fs per stage, total of 50 stages, initial temperature 500° K, final temperature 300° K, 1000 steps). CVFF force field and default values for all other parameters were used.

Binding energies were calculated each of several structures generated during the docking studies. Values of the binding energy $\Delta E_{binding}$ were calculated as the difference between the potential energy of the complex ($E_{complex}$) and the potential energy of the ligand ($E_{ligand}$) and receptor ($E_{receptor}$). (52,53) Binding energy calculations were performed using the Energy Analysis macro within the Discover_3 module.

**Receptor Binding Studies. In vitro competitive binding assay.**

The compounds were screened for their affinity for the ERα-LBD isolated from BL 21 cells that over-expressed the 33kDa PER-23d ERG vector. The cells were induced with 0.6 mM isopropyl-β-thiogalactopyranoside for 3h at RT, pelleted by centrifugation, frozen and stored at –75 °C. The cells were thawed, and lysed by sonication (4X20 sec) in four volumes of lysis buffer (50 mM Tris, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 M urea, pH 7.4) several times. Clarified fractions, obtained at 30,000 x g for 30 min were pooled, assayed for receptor binding, diluted to 50 nM in ER and 100 μL aliquots were frozen and stored at –75 °C until ready for use. Then 80 μL of the ERα-LBD- containing extract was incubated with 10 μL of 10 nM 6,7-[H-3]-estradiol (specific activity = 51 Ci/mmole) and 10 μL of either buffer, unlabeled estradiol or test ligand in 100 μL total volume. The final concentrations were 1 nM 6,7-[H-3]- estradiol, 2 nM unlabeled estradiol, (using 200 nM estradiol to define specific binding) and 0.5-5000 nM of the test ligand. In all cases, 10 μL of each incubation solution was removed for assay of the actual initial concentration of [H-3]-estradiol and the remainder was incubated at 2 °C or 25° C for 18 hours. After incubation, 100 μL of dextran coated charcoal suspension (fines removed) was added to adsorb the unbound [H-3]-estradiol, incubated for 10 min, centrifuged , and 100 uL samples were taken from the supernatant fraction for assay of radioactivity. The results were calculated and plotted as % specific binding as a function of log of competitor concentration using the best fit equation for the binding inhibition to define 50% inhibition level. The relative binding affinity (RBA) was calculated as 100 times [E]/[C], where [E] was the concentration of unlabeled estradiol needed to reduce the specific binding of [H-3]-estradiol by 50% and [C] was the concentration of test ligand needed to reduce the specific binding by 50%.

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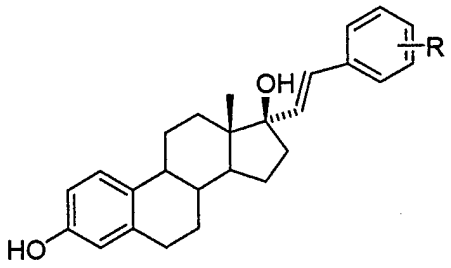
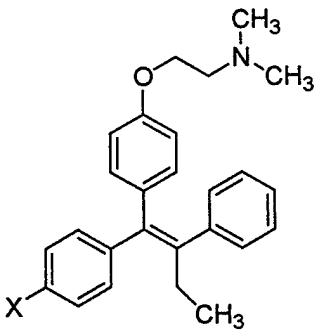


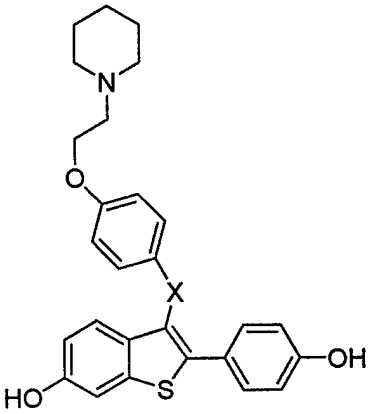

| | R= | 4 | 5 | Method |
|---|---|---|---|---|
| a | H | 25% | 92% | I |
| b | OH | 21 | 89 | I |
| c | CN | 50 | 80 | I |
| d | $CH_3$ | 59 | 60 | I |
| e | $COCH_3$ | 89 | 83 | I |
| f | $CO_2CH_3$ | 65 | 70 | I |
| g | $CO_2H$ | - | 91 | I |
| h | $CF_3$ | - | 49 | II |
| i | $OCH_3$ | - | 36 | II |
| j | F | 37 | 97 | III |

Table 1. Yields of compounds



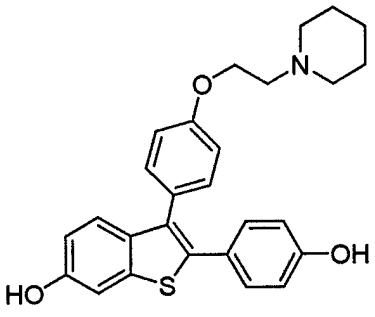
X = H, Tamoxifen

X = I, Idoxifene

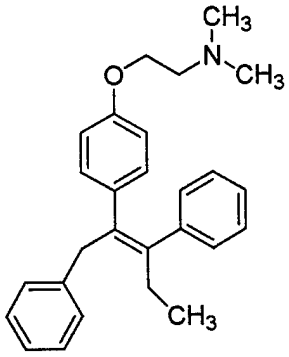


Raloxifene X= CO

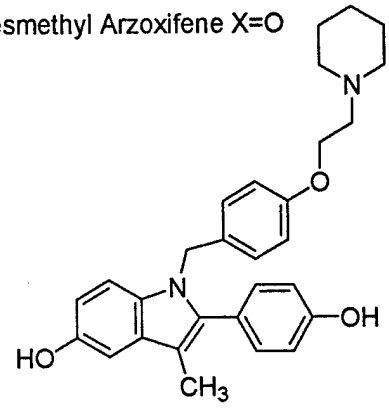
Desmethyl Arzoxifene X=O



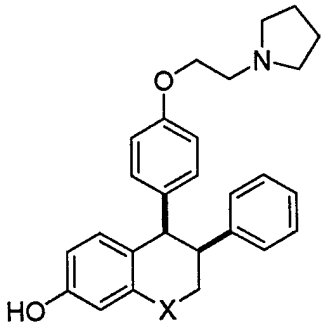
Tamoxifen-Raloxifene Hybrid



Flexible Tamoxifen analogs

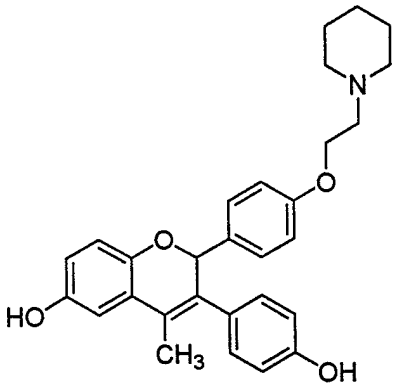


ERA-923



X = $CH_2$, CP-336156 (Lasofoxifene)

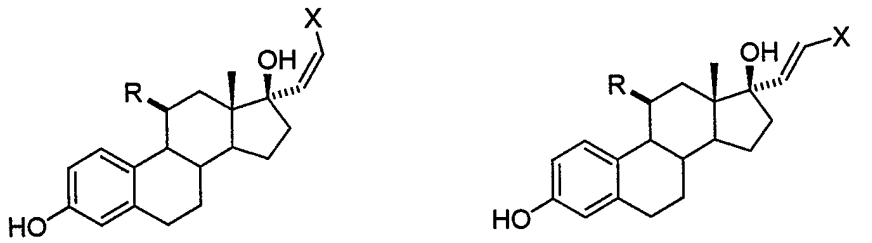
X = O, NNC 45-0781



EM-652

Nonsteroidal Approaches to Anti-estrogens

Initial Substitued 17α-E/Z-Vinyl Estradiol Derivatives




R = H, $OCH_3$, $CH_2CH_3$, $CH{=}CH_2$ X = Cl, Br, I, $C_6H_5$, $SC_6H_5$, $SeC_6H_5$

Figure 2

Rationale for selection of E/Z-substituted phenylvinyl estradiols

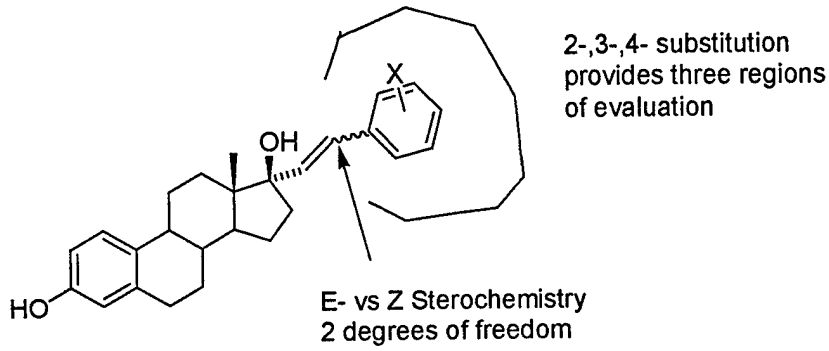
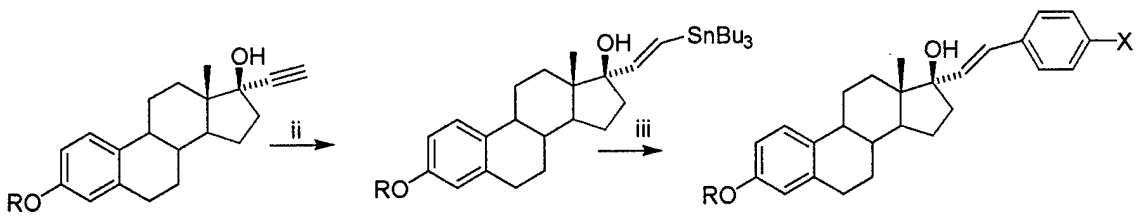



Figure 3




1 R = H

2 R = $CH_3CO$ — i

3 R = $CH_3CO$

4 a-f R = $CH_3CO$

5 a-f R = H — iv

v

5g

i. $Ac_2O$, Pyridine

ii. $Bu_3SnH$, $Et_3B$, THF

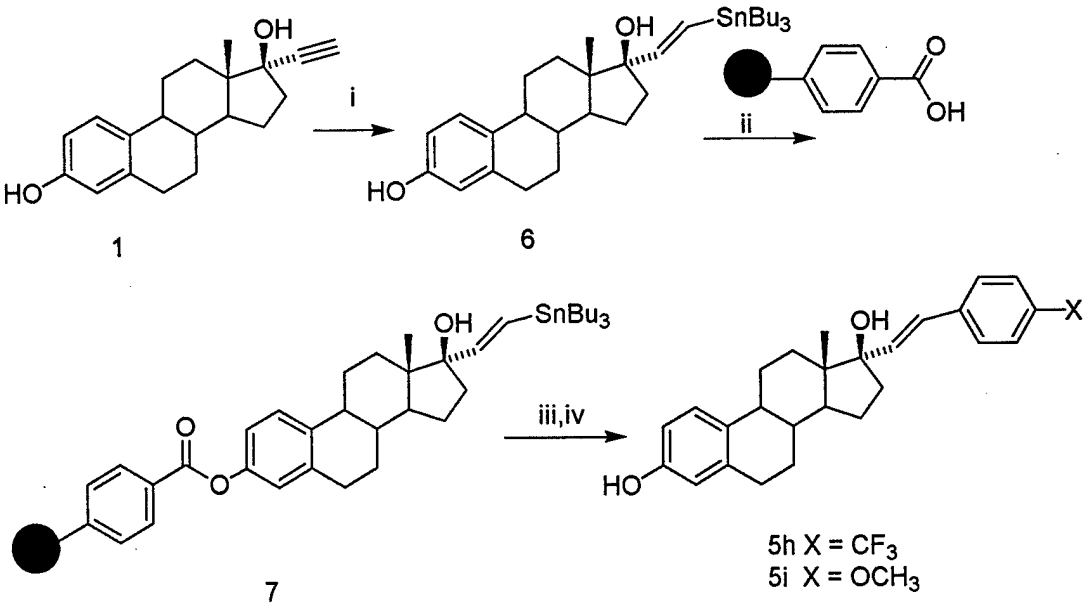
iii. $[(C_6H_5)_3P]_4Pd$, $IC_6H_4X$, Toluene

iv. $NaOCH_3$, $CH_3OH$

v. NaOH, $CH_3OH$

a X = H
b X = OH
c X = CN
d X = $CH_3$
e X = $COCH_3$
f X = $CO_2CH_3$
g X = $CO_2H$

**Scheme I.** Solution phase synthesis of estrogens.



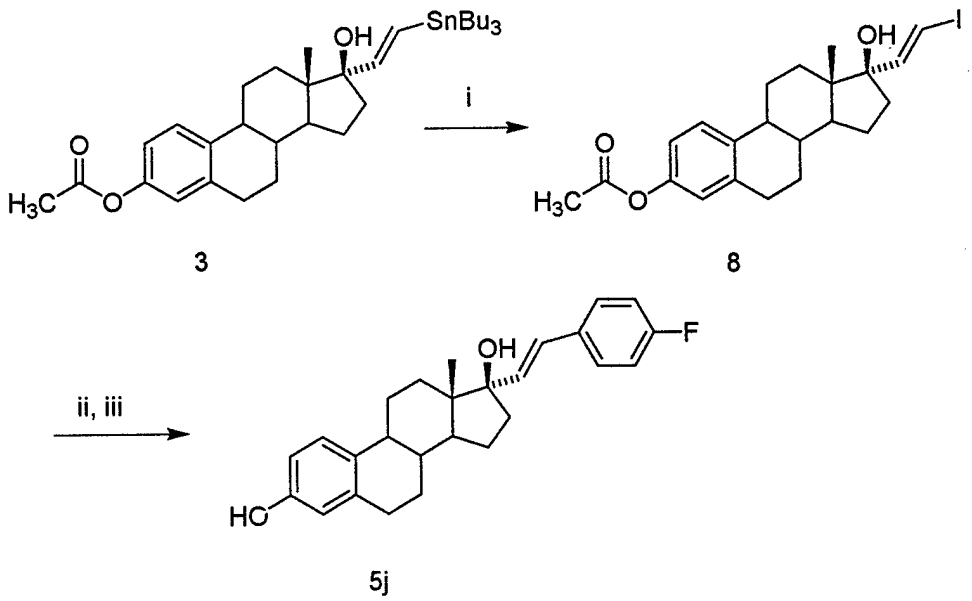

i. $Bu_3SnH$, $Et_3B$, THF

ii.CDI, THF

iii. I-$C_6H_4$-X, $[(C_6H_5)_3P]_4Pd(0)$, Toluene

iv. $NaOCH_3$, $CH_3OH$

**Scheme II.** Solid phase synthesis of estrogens




i NIS, $CHCl_3$

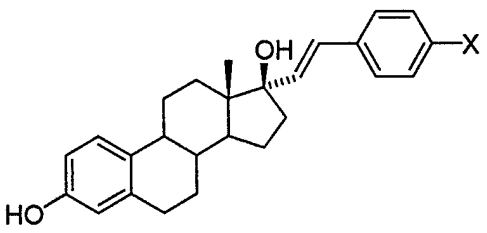
ii. $Pd_2(dba)_3$, $FC_6H_4$-$B(OH)_2$

iii. $NaOCH_3$, $CH_3OH$

**Scheme III.** Suzuki synthesis of estrogen

Table 2. Receptor Binding

| | RBA | 2°C | 25°C |
|---|---|---|---|
| a | H | 16 | 9 |
| b | OH | 21 | 25 |
| c | CN | 9 | 27 |
| d | $CH_3$ | 10 | 18 |




| | formula | calculated %C | %H | found %C | %H |
|---|---|---|---|---|---|
| 5a X=H | $C_{26}H_{30}O_2$-0.5 $CH_3CO_2C_2H_5$ | 80.38 | 8.13 | 79.87 | 8.30 |
| 5b X=OH | $C_{26}H_{30}O_3$-0.5 $CH_3CO_2C_2H_5$ | 77.38 | 7.89 | 76.92 | 8.01 |
| 5c X=CN | $C_{27}H_{29}O_2$-0.5 $CH_3CO_2C_2H_5$ | 7.55 | 7,45 | 79.05 | 7.43 |
| 5d X=$CH_3$ | $C_{27}H_{32}O_2$-0.5 $CH_3CO_2C_2H_5$ | 80.57 | 8.36 | 81.24 | 8.54 |
| 5e X=$COCH_3$ | $C_{28}H_{32}O_3$-0.5 $CH_3CO_2C_2H_5$ | 78.26 | 7.83 | 78.00 | 7.94 |
| 5f X=$CO_2CH_3$ | $C_{28}H_{32}O_4$ | 77.78 | 7.41 | 77.20 | 7.87 |
| 5g X= $CO_2H$ | $C_{27}H_{30}O_4$ | 77.48 | 7.22 | 77.02 | 7.54 |
| 5h X=$CF_3$ | $C_{27}H_{29}F_3O_2$ | 73.30 | 6.56 | 73.36 | 6.79 |
| 5i X= $OCH_3$ | $C_{27}H_{32}O_3$-0.5 $CH_3CO_2C_2H_5$ | 77.64 | 8.08 | 77.20 | 7.78 |
| 5j X=F | $C_{26}H_{29}FO_2$-0.5 $CH_3CO_2C_2H_5$ | 77.03 | 7.61 | 76.65 | 7.95 |

e $COCH_3$

53 60

| | | | |
|---|---|---|---|
| f | $CO_2CH_3$ | 18 | 26 |
| g | $CO_2H$ | 1 | 1 |
| h | $CF_3$ | 5 | 8 |
| i | $OCH_3$ | 36 | 32 |
| j | F | 24 | 22 |